

# nature

Volume 248

April 19, 1974

## Lessons from Ethiopia

WE publish in this issue a report by an Oxfam-sponsored team on what can be learnt from the Ethiopian drought of last year. This is very much a first-hand report. Mr Mason and colleagues were in the area for much of the critical time last autumn when relief efforts, including their own, were turning the tide of fatalities. They are too modest about the success of the high energy food devised at Cambridge which undoubtedly brought many children back to health from the verge of death, but the greater achievement of this group and of many other devoted workers will come if their clearly spelt out recommendations are taken seriously and acted upon. It is at least highly encouraging that they report that a framework for relief can be based on established principles. An exotic or technologically complex solution is the last thing needed.

What is the prospect for 1974? Ethiopia has two harvests the first of which comes after the light spring rains. The failure of these last year led to the crisis; this year they seem to be much better in general, although the lack of seed and oxen will ensure that the harvest is small. In addition some first results from longer term aid will soon begin to prove their worth—road building and irrigation projects have made some progress. Nevertheless there are some fearfully large problems remaining. Perhaps the greatest of these is information flow.

Everybody one speaks to about Ethiopia (and this is hardly just an Ethiopian problem, although it is particularly severe there) is concerned about the lack of data. This was epitomised recently by the conflict between a Norwegian missionary's report that the south of the country was then in distress and a BBC reporter's failure to find any such situation. It is clearly inadequate that the forays of individual journalists should form the basis on which the world is alerted, and the solution is not one which poses vast technical problems, although it may well pose political ones. The sort of monitoring that nutritionists would like to see, the sort of information that would give adequate meteorological coverage, the sort of human animal and crop statistics that would give early warning of danger are such that a trained village postmaster could provide in many countries. Of course, there are no such people as yet in Ethiopia, nor are there means of communication. But it is surely not beyond the wit of a country that runs its own airline and uses computers in government to devise a suitable permanent reporting scheme. Surely in this it would have the support of international relief agencies.

Another widely mentioned obstacle to long term progress is the problem of land use and tenure. Much of

Ethiopia's fertile land is heavily overfarmed, largely because it is parcelled up into uneconomically small units, many of which are rented out at extraordinarily high rents. Often more than 50% of the produce from this land goes in rents. The Emperor and his family, it is said, own nearly half the land in Ethiopia, and the Church a further quarter. Clearly no rational approach towards self help can ignore this source of much harm. This is one of the issues behind recent political agitations in Addis Ababa and it is difficult to see it going away spontaneously. It is to be hoped that foreign aid-giving bodies with political clout will continue to raise this matter whenever future aid is being discussed.

And what lessons are there for the aid-giving bodies themselves? It has been alarming to learn that the disaster which only broke on the world in October 1973 was known as early as April 1973 to many people who were constrained by political sensitivities to respond in a low key way. It was by no means only those in the Ethiopian government who knew, although it seems that it was they who through ignorance or touchiness underplayed the expected scale of the disaster. Nevertheless the prospect of the tragedy was known in Britain in April and even without the starkness of Mr Dimbleby's television programme, it would surely have been possible to mobilise much financial and logistic support months earlier. One has great hopes that Mrs Judith Hart, the new British Minister for Overseas Development, will look very carefully at this question. She comes to the ministry with a good reputation and a burning conviction.

She might also with profit look carefully at the ways that other countries handle aid in the field. There are tricky problems of liaison and collaboration between government and private agencies. Some have spoken well of the German approach to the operation. It is clearly a field in which all can still learn much and, one hopes, can learn quickly and without worrying about loss of face and demarcation of duties.

## 100 years ago



THE Chair of Chemistry in the University of Glasgow is vacant. We hope the Home Secretary in filling up the vacancy will, in the spirit which urged the late Mr. Langworthy to make the magnificent bequest above referred to, show by the appointment he makes the appreciation in which he holds original research. It is now high time that it should be distinctly understood that no man deserves to be appointed to a Chair of Science in any of our Universities unless he has shown that he has that knowledge of his subject which can only come from original investigation.

From *Nature*, 9, 490, April 23, 1874.



# The wind of hydrogen and of change blew gentle, clean and persistent at Miami...

*"There was the sniff in the air of American technology on the move; much more will be heard of hydrogen before long." Professor R. W. Cahn, of the School of Applied Sciences, University of Sussex, gives his impressions of a recent conference on the 'hydrogen economy' held in Miami.*

POLITICIANS and oil sheiks alike think of fuels as means of generating energy: another way of regarding them is as a means of storing energy. This approach was very much to the fore in the world's first conference on the 'hydrogen economy', organised last month by the University of Miami, with the backing of the National Science Foundation, amid the lush pleasures of the Playboy Plaza at Miami Beach. The international meeting was attended by over 700 participants, more than four times the number expected even by the buoyant organisers. Those attending were divided between the aficionados—and one of the opening speakers truly presented the adherents of the hydrogen economy as members of a quasireligious cult—and a large number of mild sceptics who were sufficiently intrigued to want to see how seriously the topic should be taken. The most popular question while sipping drinks among the bunny girls was: "And why are you here?"

Hydrogen is not a natural fuel on earth. It must first be made, most conveniently from water, and then turned back to water, generating heat (or electricity directly in a fuel cell). Since neither generation nor combustion is perfectly efficient, the cycle  $\text{H}_2\text{O} \rightarrow \text{H}_2 \rightarrow \text{H}_2\text{O}$  only makes sense from three aspects: it offers a compact means of storing and transporting energy which is not conveniently used at the site and time of liberation; it provides a possibility of creating a transport-fuel with very high energy content per unit mass; and it provides one possible substitute to pass through the insatiable natural gas pipelines of North America.

Nearly 100 papers (available in a preprint volume from the School of Continuing Studies at the University of Miami) were presented broadly on the following themes: generation of hydrogen by means of nuclear, solar or geothermal energy, and specifically by electrolysis or thermochemical cycles with catalysts, or by steam reforming of carboniferous fuels; storage of hydrogen as liquid or, more especially, in thermally decomposable hydrides; transport of hydrogen through pipelines, including problems of embrittle-

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ment by hydrogen; hydrogen as a fuel for cars and aircraft; the relative cost of hydrogen and other fuels, particularly methane, for various uses. Indeed, the conference was notable for the scrupulous care devoted to the economics of the various patterns of hydrogen production and use. The many attempts at costing covered a moderately wide spectrum, and one author's assumption was on occasion disproved by another author's technical innovation. Thus, one

of the numerous papers on thermochemical cycles, by R. E. Chao (University of Puerto Rico) and K. E. Cox (University of New Mexico), concluded that "thermo-chemical hydrogen via a series of closed chemical cycles and nuclear heat would cost \$1.50–2.35 per million BTU, whereas hydrogen from electrolysis/nuclear power would cost \$4.60–6.28 per million BTU" (compared with present jet fuel costs of \$1.70 per million BTU). But Chao and Cox assumed electrolyser capital costs at \$200 per kW, whereas L. J. Nuttall, A. P. Fickett and W. A. Titterton of the General Electric Company (GE), in their very impressive paper on hydrogen generation by electrolysis with solid polymer electrolyte,

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estimated capital costs which might be as low as \$60 per kW and overall hydrogen costs in the range of \$2–3 per million BTU (excluding liquefaction costs), which is distinctly interesting in the medium term. The GE paper was a good example of high technology transfer, since it described a thoroughly engineered process originally designed to make tonnage oxygen for the space programme, with hydrogen going to waste; this could easily be turned on its head. The GE technique effectively separates the two gases, permits extremely high current densities and, very important, gets by with exceptionally low loadings of noble metal catalyst. Ordinary commercial electrolyzers cannot afford to use catalyst and thus have to operate very inefficiently.

The numerous papers on thermochemical cycles left an overall impression of a technologically very difficult method, dependent on extremely efficient recovery of catalyst and with many uncertainties as to overall efficiency of energy conversion. It is also remarkably difficult to decide on an optimum series of chemical cycles for investigation: even a computer-aided search of possible cycles, by J. L. Russell and J. T. Porter of the General Atomic Company, gave conclusions which are hard to evaluate. One listener was left with the impression that electrolysis scores on points over thermochemical cycles, until the end of the century at least. This view was not weakened by a very interesting paper by J. B. O'Sullivan and others of the United States Army, who described a detailed and hitherto unpublicised feasibility study of a mobile hydrogen generator for field use, centring on a thermochemical cycle. The full details of this are to appear in the final proceedings of the conference, to be published by Plenum Press.

Opinions were violently divided on hydrogen as a fuel for internal combustion engines. E. M. Dickson and colleagues of the Stamford Research Institute presented a closely argued critique, with pessimistic conclusions, of the use of hydrogen to fuel 'personal vehicles'. An enthusiast, R. E. Billings of the Billings Energy Research Corporation (many small companies were represented) however described in optimistic detail his own experience of operating a fleet of hydrogen-fuelled motor-caravans, and he also claimed considerable



operating data of a converted passenger car working interchangeably with hydride or cryogenic storage.

Papers dealing specifically with hydrogen storage in cars were particularly interesting. Several discussed the detailed engineering design of hydride storage beds, mainly with reference to  $\text{FeTiH}_{1.6}$  and  $\text{MgH}_2$ . The first of these is heavy but decomposes at low temperatures; the second is light but needs to be heated to over  $600^\circ\text{C}$ . Billings explained how an optimum (i.e. lightest feasible) combination of the two hydrides could be used, with as much  $\text{MgH}_2$  as could be sufficiently heated by the exhaust gases of the engine, while hydrogen was liberated from the other hydride by means of the engine-cooling fluid. Considerable and sophisticated attention was paid to the engineering design of hydride beds for fastest possible charging and discharging for instance by G. J. Powers and D. C. Cummings of MIT. The kinetics of hydride formation or decomposition may be limited either by heat flow or hydrogen diffusion; the consensus appears to be that in the best designs, heat flow is limiting. Complete charging of a hydride bed in five minutes was claimed by one speaker; this is unlikely to be bettered.

Overall, the technology of hydrogen-fuelled motor cars seemed fairly promising; as several speakers pointed out, however, hydrogen-fuelled cars (like hydrogen as substitute natural gas) would probably require a huge discontinuous change in distribution over the whole of a large area all at once; it could scarcely come by easy stages.

Methanol, which can be made by a hydrogen route, received attention as a possible automotive fuel. In an impressive paper modified by some last-minute findings, T. B. Reed and R. M. Lerner of MIT dealt with the potential for progressive lacing of petrol with synthetic methanol, as methanol gradually becomes cheaper relative to oil. The prospects are promising, according to this work, that increased methanol content will so improve anti-knock properties that the lead content can be considerably lowered or perhaps even eliminated. The conflict of all-or-nothing versus progressive introduction of new fuels was one aspect of the 'social' approach to the hydrogen economy which several speakers essayed. Dickson's paper from Stanford was outstanding in this connection. He analysed the magnitude of the impact of various changes inseparable from a hydrogen-fuelled car economy and also examined which groups of people (drivers, mechanics, fuel dispensers, fuel distributors, fuel manufacturers) were affected. His histori-

R. R. Adt of Greenwell and M. P. Swain of the University of Miami. Technology and economics are but two sides of a triangle, of which politics forms the base.

The long paper by T. C. Cody of West Virginia University on the hydrogen economy and (American) law was particularly thought provoking. Cody foreshadows in-

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creasing "federal preemption" over the states in safety and environmental legislation and a progressive sharpening of legal limitations on energy use: eventually he foresees a law of allocation to replace the present 'crazy-quilt of contradictions, cross-purposes, omissions, overlap and duplication.'

On hydrogen for aviation, opinions were also divided. R. A. Lessard, an economist of the United Aircraft Research laboratories, in a separately circulated paper, was sceptical on purely economic grounds, whereas P. F. Korycinski and D. B. Snow of NASA were optimistic on the basis of a detailed technical analysis. The larger the aircraft and the greater the range, the greater is the economic advantage of using liquid hydrogen. One listener at least was left with the clear impression that aviation will be the first major user of hydrogen, well before the century is out. This view was shared by a severe critic of the hydrogen economy, P. N. Ross, a vice-president of Westinghouse, who was utterly convinced that nuclear electricity, distributed as such, would indefinitely defeat the use of hydrogen—except for aircraft.

There is no space to do justice to the group of papers on solar and geothermal energy (the latter seems particularly promising in an American context). The conference showed very clearly its recognition that hydrogen is merely a means of storing energy, not creating it. Some new, cheap solar cells were developed and evaluated, the engineering aspects of a mile-square servo-controlled mirror array and boiler tower were analysed, there was much to-do about large floating platforms making use of thermal gradients in the oceans (a form of indirect solar energy) and wind power came in for some detailed analysis, not least in connection with a self-contained domestic system using only established technology, including energy storage by electrolysis and later use of the hydrogen. This intriguing study was by L. W. Zelby of the University of Oklahoma, where the wind is gentle but persistent.

Indeed, the wind of hydrogen and of change blew gentle, clean and persistent at Miami. The fact that hydrogen is almost pollution-free was much commented on (but see *Nature* for March 29, page 458); the name of the Environmental Protection Agency was much mooted and an International Association for Clean Energy was founded at the conference. There was the sniff in the air of American technology on the move; much more will be heard of hydrogen before long.

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cal parallels—for instance, the disincentive to the spread of diesel-fuelled passenger cars which he identified with the mild inconvenience of locating supplies of diesel fuel—lead him to argue strongly that while quite major changes in engine design, such as the Wankel engine, can be quite readily accommodated, relatively modest changes in nature of the fuel, end especially of fuel distribution, will constitute major blockages.

One advantage of the kind of analysis offered by Dickson is that it can alert technologists to needs that otherwise might be underestimated or even ignored. If it is accepted that the introduction of a hydrogen-only car would come up against particularly intractable socio-political resistances, then there is a strong incentive to develop an engine that can run on either of two alternative fuels—such as the hydrogen/methanol engine described at the conference by

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### Fuel prices and the energy crisis

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| Fuel        | Price before the<br>energy crisis<br>(\$ per million BTU) | Estimated<br>1974 price |
|-------------|-----------------------------------------------------------|-------------------------|
| Natural gas | 0.25                                                      | 0.70                    |
| Fuel oil    | 0.32                                                      | 1.80                    |
| Coal        | 0.20                                                      | 0.58                    |

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# international news

EVERY year 7,000 people in Britain die from kidney disease. Five thousand of these die simply because a cure for the chronic form of the disease has not yet been found; the remaining 2,000 fall into the category that are likely to respond to a kidney machine or to a transplant, but they die because there are not enough machines or cadaver (donor) kidneys to go round. In the short term, a rapid turnover to transplantation permits the acceptance of more patients into dialysis units and so an immediate and urgent need is for many more cadaver kidneys.

Twenty-three transplant units have been set up in the United Kingdom since the Medical Research Council advised the Department of Health and Social Security in 1967 that renal transplantation had 'passed beyond the research stage'. Surgeons now perform more than 500 transplants between them every year; the survival rate has continued to climb steadily and kidneys have proved themselves the most suitable of all transplanted organs. But the demand for kidneys far exceeds the supply, and it is a lack of understanding and cooperation among doctors themselves that is largely to blame.

"Organ donation" said a report issued by the Royal College of Physicians (RCP) in 1972 "involves the doctors caring for the patient in a considerable amount of extra work and trouble. They must talk to the relatives, consult the coroner and make numerous telephone calls. Most of these patients are not now considered as organ donors because the question is never raised. Yet this negative attitude is the major limiting factor in the expansion of a renal transplant service . . . Most important of all," the report concludes, "is for there to be more cooperation within the medical profession so that the donor organs are not wasted."

Some London hospitals were already engaged in a kidney-sharing programme and as a result of their example and the RCP's report, the Department of Health set up in 1972 the National Organ Matching Service based at the Southmead Hospital in Bristol. The names of patients waiting for kidneys are kept on a computer and when donor kidneys become available the most suitable recipient is selected. The following year, the Department issued 2,000,000 donor cards, which although never intended as legal documents were designed to enhance the public's aware-

ness of the kidney problem. But neither the donor cards nor the matching service has provided the results everyone was hoping for.

There were no more kidneys available last year than in 1972; and surgeons are particularly disappointed at the comparatively small number of matches effected by the Bristol centre. But there

own success rate is very high and yet a consultant from another department in his own hospital walked through his ward recently and asked whether he had anyone still alive after a transplant. Mr Barnes spends every spare minute visiting hospitals in his area reminding doctors and surgeons about kidneys. But there are still a number of hospitals from which he has never even had the offer of a kidney.

A member of another kidney team recently accused doctors of being reluctant to ask relatives for permission to take kidneys from newly dead patients. Some doctors, he said, take it as a personal failure when a patient dies, but they should appreciate that a kidney could save another life. "I know it seems callous," he said, "but we have to educate our accident colleagues that it is necessary to act quickly when a suitable patient dies."

Michael Bewick, who transplants kidneys at Guy's Hospital, London, says it is "quite appalling" to have to ask relatives for permission to retrieve kidneys soon after bereavement. He says the Department of Health should introduce an 'opting in' system where everyone would be formally asked to consider becoming a potential donor. The tissue details of all those who agreed would be kept on the computer at the Organ Matching Centre and when anyone died suddenly or was suffering from a terminal condition, the doctor would simply check if his patient was on the computer's list.

Roy Calne, Professor of Surgery at Cambridge, favours an 'opting out' system whereby everyone becomes a potential donor unless he registers his dissent. Professor Calne says a change in the law and more collaboration within the profession are the best ways of meeting the ever increasing demand for kidneys. "But", he says, "I doubt whether there will be a significant improvement until the public puts pressure on both the government and the medical profession. And that won't happen until the results of kidney transplantation are well enough known."

The Department of Health and Social Security is unlikely to introduce either an 'opting in' or an 'opting out' system in the near future, although Dr. David Owen, Under Secretary of State for Health, said recently in a Commons written reply that such a scheme was under consideration. The department is, however, launching a publicity cam-

## Transplants: the failing machinery

Robin Laurance



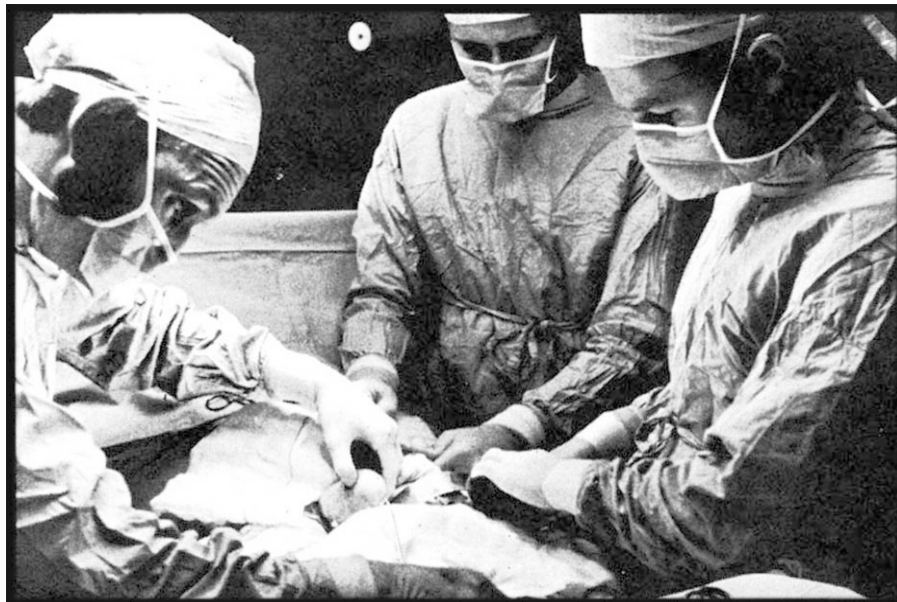
is special concern about the reluctance of some hospitals to share out the kidneys which are not suitable for their own patients. Last year, for instance, the Hammersmith Hospital received 38 kidneys from the pool but contributed only six. St Mary's Hospital took 31 and contributed 8; Queen Elizabeth's Hospital in Birmingham, on the other hand, received 38 and contributed 74.

In addition to an apparent lack of cooperation between the surgeons themselves, there is an even greater gap between the surgeons and the rest of the profession. Mr Anthony Barnes (the surgeon involved in a recent controversial transplant operation at Birmingham, said to have been started before the donor was dead) says the main problem is ignorance and that the majority of doctors simply are not aware that transplants are such a successful treatment for kidney failure. His

paign with 30,000 posters to be sent to hospitals and doctors' surgeries reminding people that when they die, their kidneys could save other people's lives. But without a change in the law, any positive response will only cause frustration all round.

So the problem comes back to the doctors themselves, who have to make the most of the kidneys that come their way. One of the headaches here is defining the moment of death in the donor—a problem that has become even more difficult in recent months with the increasing use of ventilators. For a successful transplant, a kidney must be extracted within an hour of death and where the patient is being kept 'alive' artificially by ventilation the doctor (and in transplant cases, two doctors are required to certify death) must decide when the brain has died and when the ventilator has passed the point at which it will invoke any involuntary response in the patient.

Anthony Barnes maintains that many doctors are still only learning when to turn the ventilator off. Some, he says, do an injustice to relatives by allowing their patients to rot with a brain that is never going to work again. "And the kidneys become rotten too," he says, "and that's an awful waste." And to rub in the dilemma facing doctors, the coroner at the Birmingham inquest involving Mr Barnes, told the jury that



*A patient receives a transplant kidney at Guy's Hospital, London*

there was "considerable dissention among pathologists he had questioned about when a person was dead".

Doctors abroad involved in kidney transplants already have fewer problems. In France, and in some parts of the United States, brain death is accepted as a clear index of death, which means that much fresher kidneys are available from donors who are still breathing. (It also, of course, means

that many patients are spared lives as cabbages.) The Americans also have the Uniform Anatomical Gift Act which has made donor cards legal documents.

And in Scandinavia the law says a doctor can take what he likes from a corpse, with no questions asked. Clearly, legislation along these lines would make life easier for transplant surgeons working in Britain.

THE pay of government scientists in Britain will continue to be determined by reference to that of scientists in industry rather than by comparisons with other branches of the Civil Service if the recommendations of the Pay Board on Science Group pay are accepted as policy.

About 16,000 members of the group are affected by the report of the board, which appeared after a protracted dispute over the criteria for determining their salaries. The scientists, represented by the Institution of Professional Civil Servants (IPCS), were loth to accept a pay research exercise referring to the rates of scientists in industry because, it was argued, the government was such a large scale employer that industrial rates were themselves determined by the level of pay in the public sector, and so any comparison would be prey to an illogical circularity.

The report says, however, that the process of pay research is not invalidated by these special circumstances, and that they can be taken into account, either by the Pay Research Unit (PRU) in making comparisons or where this is not possible, by the parties involved in negotiation.

The IPCS had pointed out in evidence to the board that scientists

## Pay Board reports on scientists

had not had a substantive pay review since January 1971, when there was a measure of parity between their rates and those of comparable grades in the Civil Service Administration Group. By January this year the scientists had fallen behind, at the scale maxima, by as much as £880 at Principal Scientific Officer (PSO) level, and £731 at Higher Scientific Officer level.

According to the IPCS new rates should have been determined, according to a principle of the Priestley Commission, by internal relativities, using the Professional and Technology Group as a comparison, rather than the Administration Group, as in the past. But the only point at which the Pay Board leaned towards internal relativities was in its suggestion that PSO rates should at both ends of the scale (currently £3,715 and £4,895) be no more than 5% away from the rates for their counterparts in the administrative grades. As a corollary, the report recommends that the maximum of the Senior Scientific Officer scale (now £3,895) should be adjusted

if pay research does not produce an appropriate differential with the minimum of the PSO scale.

Both these adjustments would take into account what the report calls the "unquantifiable" factors of individual merit and the differing career pattern of government scientists, who tend to go on working in research and development long after the age at which their industrial counterparts have moved on to greener pastures. About 5,500 scientists would be affected by this suggestion.

The remaining 10,500 members of the Science Group will have to be content with pay research, although there is a recommendation that procedures for the exercise should be reviewed jointly by the scientists, the Civil Service Department and the PRU.

The IPCS said it was "severely disappointed" with these formal recommendations, which would take a long time to implement (January 1976 has been suggested as the earliest date by which the exercise could even get under way). But the union adds that, given goodwill on the part of the government, there is no reason why an agreement should not be reached and it has entered an interim claim, based again on internal relativities.



## Berkeley's line on PWRs

Eleanor Lawrence

THE Nuclear Laboratories at Berkeley in Gloucestershire provide the Central Electricity Generating Board (CEGB) with nuclear research facilities and expertise without parallel in any electricity utility in the western world according to CEGB Board Member Mr Donald Clark. At present, with the CEGB's controversial proposal to buy American-designed pressurised water reactors (PWRs) very much in the air, evaluating and verifying work on the safety of these reactors is one of Berkeley's most pressing problems.

According to Dr Bryan Edmondson, the director, very recent work by Westinghouse on the PWR pressure vessels indicates that there may be less danger than feared of the catastrophic crack propagation emphasised by opponents of PWRs. At the temperatures at which the reactors operate, defects in the steel pressure vessels would probably propagate by plastic deformation and not by sudden crack propagation. In a half-size model with 6-inch walls (compared with 12–15-inch walls in the full-size vessel) Westinghouse tests have shown that this does in fact lead to a 'leak-before-break' situation.

Dr Edmondson emphasises that this, although comforting, is not the sort of information on which the safety case for the reactor could be based. Quality tests during manufacture, overpressure testing before service and regular in-service inspections are the best lines of defence. Also, as the vessels need not be fully pressurised until a temperature of 300° C, they can be carefully controlled during the heating up process through temperatures at which the danger of catastrophic crack propagation is greatest. The pressure vessels are also more accessible to repair and inspection than the innards of the gas-cooled reactors and, as technology has been developed to deal with crack detection in those circumstances, Dr Edmondson is hopeful that it will not prove too big a problem with PWRs.

Commenting on the memorandum sent to the Select Committee on Science and Technology by Sir Alan Cottrell, highlighting the problems of pressure vessel safety, Dr Edmondson says that he could not disagree with Sir Alan and had been giving similar advice to the Board, but that the results of the Westinghouse tests had not been available then.

Berkeley is also evaluating the American calculations on what would happen to the pressure vessel in the case of a loss-of-coolant accident when it would be subjected to a shock of cold water.

The laboratories are now half-way through the stress analysis and so far have come up with the same reassuring answers as the Americans, whose calculations have been accepted in the safety case in the United States.

Dr Edmondson emphasises that because the PWR matter is so controversial much more evaluation and verification of already published results, which would normally be taken on trust, is having to be done. But he does not expect that the findings will differ drastically from previous work.

## Russia's Mars probes

from our Soviet Correspondent

THE ANNOUNCEMENT of the partial failure of the latest Soviet Mars experiment—the probes Mars-4 to Mars-7—was one of the less auspicious features of the Jubilee General Meeting of the Academy of Sciences on March 5th 1974. Already it was known that the breaking motors of Mars-4 had failed to operate so that it achieved only a close by-pass at 2,200 km. Nevertheless some good photographs had been transmitted from Mars-4, and Mars-5 was satisfactorily in orbit investigating the planet, its atmosphere and ambient space and two further probes were still on their way.

However, Mars-6 and -7, which reached the vicinity of the planet on March 12th and 9th respectively were unable to retrieve the hopes of the programme. Although the official Tass announcement called their flight "an important step in the investigation of Mars", the news was depressing. The two probes had carried descent capsules, which would investigate the surface and relay data to the orbiting section for re-transmission. There had even been speculation (evoked by a Pravda article on future plans for planetary research) that one of them might be carrying a roving "Marsokhod". However, radio contact with the descent stage of Mars-6 was lost when it had reached "the immediate vicinity of the surface", and a failure in the on-board systems of the descent stage of Mars-7 after separation resulted in a fly-by at 1,300 km. The "orbital" stages of these two probes apparently failed to enter aero-centric orbit, since they are reported to be "investigating the physical characteristics of space" from helio-centric orbits. So the overall design of the experiment, with four orbital stations monitoring the same data from different altitudes together with two surface probes (the previous programme used only two such stations and one surface probe), has been reduced to a single orbital station and a number of

fly-by photos, valuable though these are.

The claims in the Soviet press that nevertheless valuable results have been obtained are not however merely an attempt to save face. Photometry readings, for example, have revealed a maximum precipitation of some 60 nm—a value several times greater than that recorded by similar instruments aboard Mars-3 in 1972, which, it is suggested may indicate a different rate of release of water vapour from the crust into the atmosphere in different regions of the planet. Traces of atmospheric ozone have been noted for the first time. The existence of the magnetic field of the planet (indicated by the data of Mars-2 and -3) has been confirmed, and readings have now been obtained for the magnetic field on the dark side of the planet.

Nevertheless, a sense of disappointment is apparent and a long article in Pravda, March 20th, on the engineering problems of designing planetary probes may well reflect not only a desire to restore public confidence but also some serious re-thinking by the planners of the "hardware" situation.

## ELSE in Israel

Nechemia Meyers, Rehovot

EDITING and publishing a scientific journal is a very ordinary venture which anybody can do, and almost everybody does—at least, in the opinion of Mr Paul Nijhoff Asser, Secretary General of the International Group of Scientific, Technical and Medical Publishers. Mr Nijhoff Asser made this judgment at a recent meeting in Israel of a small but influential group of scientific communicators, the Executive Council of the European Association of Editors of Biological Periodicals (ELSE). He was commenting on a report from ELSE treasurer, Miriam Balaban, that 130,000 scientific journals are currently being produced and that the number is rising.

The European editors, who in the past have seemed anxious to demonstrate their independence of United States influences—preferring, for example, to issue their own manual of scientific writing and terminology—nevertheless invited an observer from their American counterpart organisation, the Conference of Biological Editors. Dr Karl Heumann, Executive Editor of the Federation of American Societies for Experimental Biology, took an active part in the discussions and suggested that editors should carefully consider whether to publish papers describing experiments in which the human participants were not aware of the risks they were taking.

Small though the meeting was, the Israelis present regarded it as another victory in their continuing struggle to establish their European credentials.



## Academy exposes a sham

Colin Norman, Washington

FOR years now, spokesmen for the Nixon administration have been touting the line that federal research and development expenditure are gradually being focused on programmes which will benefit society and help solve "critical domestic problems" in the United States. Such sentiments reached their zenith two years ago, in March 1972, when President Nixon sent his first, and only, message to Congress on science and technology. His statement was replete with rhetoric about a new partnership which would be forged between government, industry and the universities, to help bring the fruits of technology to the people.

But a committee of the National Academy of Engineering (NAE), the most prestigious engineering organisation in the United States, published a report last week which came close to suggesting that most of President Nixon's science and technology message has turned out to be a complete sham.

The committee states that "with a few exceptions, the vast technology developed by federally funded programs since World War II has not resulted in widespread 'spin offs' of secondary or additional applications of practical products, processes, and services that have made an impact on the nation's economic growth, industrial productivity, employment gains and foreign trade".

The committee's general thesis is that technology developed with federal funds probably hasn't been exploited for the good of the people (or at least for the good of corporate profitability) because "a plethora of structural and institutional barriers exist in the federal government and the private economy to prevent the efficient and effective utilisation of this technology". Furthermore, since there has never been a comprehensive effort to search out technology which can be usefully exploited, it is difficult to recommend policies in such a vacuum of knowledge.

The NAE's findings and conclusions are shared by many observers of federal science policy, including a few key members of Congress. It was only just over a year ago, for example, that the Senate passed, by a huge majority, a bill sponsored by Senator Edward M. Kennedy which would have given the National Science Foundation a large budget to bring federal research and development to bear on such critical problems as urban renewal, health care delivery and transportation.

The bill died because it was never approved by the House of Representatives, but the sentiment lives on. Just



MERCURY as seen by the cameras of Mariner 10. Left, heavily cratered area reminiscent of the highlands of the Moon, including a bright rayed crater. Picture taken from about 400,000 km resolves craters 120 km or more in diameter. Below, closeup picture taken from a distance of 20,700 km about 30 min before closest approach. The clearly defined central crater (about 12 km across) is a relatively new feature.



last month, for example, the Senate Committee on Science and Astronautics held extensive hearings on a bill which would provide NASA with a large helping of money to promote civilian technology. The bill is not likely to get anywhere this year because, even if it is approved by the Senate, the House of Representatives has so far shown little interest in the measure and in any case congress is likely to be occupied later this year with impeachment fever.

But there continues to be considerable feeling on Capitol Hill that there is a lot of federal research and development which could be usefully exploited if only suitable arrangements could be worked out to accomplish the task.

The NAE committee, at least, has a few concrete recommendations for removing some of the barriers to innovation which have prevented private companies from turning federally produced technology into marketable products. For a start, it suggests that the federal government should spend \$1,000 million a year in helping to promote the utilisation of technology through a variety of incentives and government programmes.

The first task should be to conduct a survey of federal laboratories, to test "the assumption that there is a substantial amount of useful federally funded technology available for beneficial, widespread secondary application."

Assuming, as the committee does, that

the survey turns up positive results, federal agencies should set up explicit programmes with specific charters for embarking on technology utilisation projects, and they should be given funds earmarked in the agencies' budgets for that purpose. Certainly, some agencies, such as NASA, have already established such offices, but the NAE committee reckons that much more could be done. But the committee is quick to point out that its recommendations in no way "imply that the federal government should become a competitor to the private entrepreneur", for the idea is that once a technology has been developed, it would be adapted, produced and marketed by private industry.

In order to stimulate that end of the process, the committee suggests that the federal government should provide a variety of incentives such as risk assurance, exclusive licences and "imaginatively bold financing to users in the public and private sectors in order to accelerate the direct implementation or to stimulate financial institutions to provide greater investment in new technology".

If all that sounds familiar, it is. Such suggestions have been made by virtually every committee which has studied the federal government's role in exploiting research and development financed by the taxpayer for the good of the taxpayer. But so far, there's little sign that anybody is listening.

# correspondence

## Newton and Kepler

SIR,—I have only just read Dr J. W. Herivel's review in your journal (*Nature*, January 18, 163–164) of the recently published variorum edition of Newton's *Principia*. There I am fathered with I. B. Cohen's view—"originally put forward [in my article on 'Newton's early thoughts on planetary motion in'] *Br. J. Hist. Sci.* 2, 117–137 (1964)"—that Newton "apparently learnt of Kepler's law of areas in 1678 and at once was able to solve the problem of Keplerian planetary motion" (Herivel's italics). Quite bluntly, I have never said anything approximating to that, with or without its emphasis, there or anywhere else.

In my 1964 article I did, to be sure, publish my (then) novel finding that nowhere in his extant early papers "does Newton make any mention of Kepler's area law"—with the qualification in a footnote thereto (page 124) that in his "deep study at an early age of Wren's . . . tract [on Kepler's problem, as published by Wallis in his *De Cycloide*, 80 (1659)] . . . it is just possible his attention was caught by the fleeting reference [there] to the area law"—and then went on to observe (page 131, note 48) that Newton possessed in his personal library a "well-thumbed" copy of Nicolaus Mercator's *Institutiones Astronomicae* (1676) where, as I wrote, "correct enunciation" of the planetary law is given. That it is, as Herivel now says, "stretching credulity to suppose that Newton had not . . . deeply pondered on this extraordinary law before 1679 [sic]" may or may not be everyone's modern reaction; but such an undocumented opinion is certainly irrelevant when, as I did, one comes to examine the available historical evidence. I know nothing of Herivel's recent scholarly activity in this area but after my own nearly twenty years of continuous study of Newton's scientific papers (including many in private possession which are not generally accessible) I will reiterate that I have as yet found nothing which establishes in any way that Newton was, before 1676, aware of the law's verbal enunciation or begins to hint that he "deeply pondered" its meaning at any time before late 1679. If Herivel knows better than I, let him state his sources.

As for the portion of Herivel's quot-

ation which he italicises, this, whatever its truth, greatly traduces the hypothesis which I formulated in my 1964 paper. There I made Newton's "sudden" appreciation of the validity of the area law in 1679 dependent, not on his presumed ignorance of the law's statement up to that time, but on his recognition of its theoretical necessity in his proof of the elliptical orbits of the planets under the condition (as Hooke laid it down in their correspondence in the early winter of 1679–80) that their undeviated motion be uniform and in a straight line. Herivel knows this very well since, on the appearance of my article 10 years ago, he mounted a hasty and often ill-considered but full-blooded attack on it in *Br. J. Hist. Sci.* 2, 350–354 (1965), in the course of which he allowed my hypothesis to be "possible [but] very improbable". That unsupported judgement is evidently now to be sanctified by similarly ruling out

### Brevity

CORRESPONDENCE stands more chance of being published if it does not exceed 350 words. The Editor reserves the right to abbreviate any item.

as "very improbable" a distortion of my original thesis which I have never held. May I ask Herivel to acknowledge as much and to retract his present aspersions?

D. T. WHITESIDE

Cambridge, UK

DR HERIVEL REPLIES: Dr Whiteside protests at my "fathering" on him the view of I. B. Cohen that Newton "apparently learnt of Kepler's law of areas in 1678 and at once was able to solve the problem of Kepler motion" (my italics). This view seems however, to have been suggested to Cohen by Whiteside's 1964 paper to which he refers immediately afterwards. This does not seem so improbable as it might otherwise appear from Whiteside's letter in the light of the following passage in his paper (page 128):

"We have said enough to justify our general contention that in the autumn of 1679 Newton was, if indeed he at all consciously then recognised its existence, still unwilling to allow Kepler's crucially important second (areal) law even an empirical place among the axioms of his astronomical thought."

I myself derived somewhat the same

impression as Cohen from a reading of Whiteside's paper. For in my "hasty and often ill-considered" paper of 1965 I said:

"to the various explanations already put forward, a new one has recently been added by D. T. Whiteside, namely, that Newton was prevented from solving the problem of Kepler motion before 1679 because up to that time he was either unfamiliar with, or had no confidence in, Kepler's second law of planetary motion".

As regards the second, italicised, part of Cohen's view, although I did not draw this conclusion myself I can quite understand Cohen's doing so. For if Whiteside's paper is interpreted as implying that Newton was unfamiliar with Kepler's second law up to the autumn of 1679, and if he required this law for his solution to the problem of Kepler motion in the following winter, then it does not seem unreasonable to conclude that it was his tardy acquaintance with this law which led to the solution of the problem.

In the light of Whiteside's letter I am quite happy to retract the phrase "This view originally put forward by D. T. Whiteside". But in fairness to I. B. Cohen, and for the sake of the historical record, I should want to replace it by the following: "this view was apparently suggested to I. B. Cohen from a reading of a paper by D. T. Whiteside".

As to the important question of whether or not Newton was consciously aware of the exact, areal form of Kepler's second law before the autumn of 1679, it seems to me that in the light of Dr Russell's findings the onus is on Whiteside to provide documentary evidence for a lack of familiarity on Newton's part. In the absence of this evidence I for one will continue to believe that Newton was perfectly well aware of the exact form of the law long before 1679, most probably from a reading of Kepler's *Epitome*. For if Dr Russell is correct in believing that this famous work of Kepler was "from about 1630–50 or beyond almost certainly the most widely read treatise on theoretical astronomy in Europe" (*Br. J. Hist. Sci.* 2, 20; 1964) then I find it very difficult to believe that it was not read by Newton. Documentary evidence is important, and without it history cannot be written, but its absence does not necessarily preclude the historian from drawing probable conclusions in other ways.



# news and views

## Should fossil hominids be reclassified?

RICHARD LEAKEY'S report in this issue of *Nature* signifies the end of the sixth field season in the Lake Rudolf area. In those six seasons important new fossil material has appeared at a rate almost too fast for scientific interpretation if not intellectual assimilation. At present, it is possible to discern two important contributions of the East Rudolf work, although a greater time perspective may indeed add further dimensions and ramifications to present interpretations of the Rudolf material.

The first and most obvious contribution has been the enormous increase in the size of the fossil sample, not only of hominid material but also of other Primates and vertebrates. The work at Rudolf has so far yielded approximately 107 specimens of fossil hominid; by comparison, Olduvai Gorge, an area noted to be very productive of fossil material, has yielded only about 40 hominids in nearly as many years. A second important result of the Rudolf work has been a recognition of the necessity to examine closely some of the earlier concepts and interpretations of human evolution. Before the 1970s the widely accepted model of human evolution suggested a slow and orderly emergence of a more advanced evolutionary grade of man from a primitive basal stock. In this way, late Pliocene-early Pleistocene australopithecine populations were seen to develop slowly into middle Pleistocene populations, generally attributed to *Homo erectus*; in fact, the process may not have been so simple.

The fossil evidence of man at East Rudolf now demonstrates a very wide morphological variation. There is clearly a large, robust-type australopithecine as seen in the cranium KNM-ER 406, mandibles KNM-ER 729, 818, 1806 and other specimens. There is also evidence of a more lightly built and perhaps more advanced group seen in the crania KNM-ER 1470 and possibly 1590, mandibles KNM-ER 730, 820, femur KNM-ER 737 and others. Certain specimens, notably the cranium KNM-ER 1805 and the mandible KNM-ER 1482, have proved difficult to assign to any currently recognised group. An interesting aspect of the Rudolf material has been the apparent absence of the gracile form of australopithecine. This type is well known from sites in South Africa and from Olduvai Gorge and now Leakey suggests that a cranium recovered during the last field season, KNM-ER 1813, may indeed represent that group at Lake Rudolf.

The variety of morphology present in the Rudolf hominid sample poses a number of interesting questions and of these perhaps the most important concerns the relationship between the advanced primitive members of the gracile groups. Does the gracile group contain generically distinct *Australopithecus* and *Homo* or does it contain only one taxonomic group with wide variation due to temporal, behavioural, sexual or geographical factors? Robinson, in placing all of the more gracile types in the genus *Homo* (*Nature*, **205**, 121; 1965), has favoured the second explanation. His classification, based largely on features associated with dental mechanisms, implied significant dietary differences between the robust and gracile groups. Leakey now suggests that there may be two gracile groups—one advanced and one with more primitive morphology, distinct at the generic

level—in addition to a large, robust group and perhaps one other. He thus suggests the existence of at least three separate hominid lineages, sympatric and contemporary, within the early Pleistocene. Such a classification imposes problems.

First, it is difficult to understand how the East African environment could provide adequate ecological space to support three or more separate hominid lineages which must have been in competition for territory and for some basic subsistence materials during a period of 2 million years or more. Leakey's interpretation of the evidence would seem to suggest an early adaptive radiation within the Hominidae but since all groups were living in presumably the same environment, it is difficult to visualise the adaptive pressures and isolating factors which are necessary to produce genetic separation in related groups. Finally, and most important, if one accepts that gracile australopithecines and *Homo* coexisted in the early Pleistocene, then one must also accept that the available sample of australopithecines does not, and indeed cannot, represent the ancestral hominid group. The gracile australopithecines are widely accepted as the basal hominid stock and yet Leakey's current classification of the early hominids implies that the known members of this group coexisted with the genus *Homo* and were in fact too late to provide the ancestral population.

The problems associated with interpretations of the Rudolf material demonstrate the urgency of the need to re-evaluate the taxonomic criteria for genera within the Hominidae. The definition of the genus *Homo*, proposed in 1964 by Louis Leakey, Tobias and Napier (*Nature*, **202**, 7) is inadequate on several grounds and failed to demonstrate adequate morphological space between the gracile australopithecines and *H. habilis*, the most primitive member of the genus *Homo*. Robinson's classification separated the robust australopithecines ("*Paranthropus*" in his terminology) from all gracile hominids ("*Homo*") on the basis of dietary and behavioural factors. Still others have suggested that all of the australopithecines should be included within a single taxa. There is still, however, no widely accepted classificatory system. The present difficulty is due partly to the inevitable incompleteness of the fossil sample and partly to the inadequacy of Linnaean systematics in dealing with evolving populations; in the particular case of the Rudolf material, Leakey's typological approach to classification adds yet another dimension to the difficulties. Palaeontologists must be dealing with evidence of evolving lineages and yet by continuing to compartmentalise this evidence into discrete taxa based on typologically orientated criteria, they are losing sight of the continuity and cohesiveness of the fossil record. Typological classification emphasises the distinctiveness of the material and must, also, therefore, implicitly suggest discontinuity in the fossil record. A more appropriate approach might emphasise functionally-based similarities and expected variability in evolving populations and would emphasise the continuity of the evolutionary process.

Leakey suggests in this issue that in view of the large amount of fossil human material now available he intends to make a review of hominid systematics. Such a review is necessary, but before it can be of practical importance, the theoretical bases of hominid palaeotaxonomy must be re-evaluated and if necessary reformulated. Without a valid and widely acceptable theoretical framework for classification of the fossil Hominidae such a taxonomic review may be premature and will only add further controversy.

From our Palaeoanthropology Correspondent

# Predicting how proteins fold up

In current and pending publications there are signs of a new, more ambitious phase in the struggle to predict the biologically active conformation of a protein from its covalent structure. Certainly there is much incentive for ambitious investigations in this field. In the right conditions, many proteins spontaneously and reversibly fold into their biologically active conformation maintained by intramolecular non-bonding forces and reducible disulphide bridges. Such observations have appropriately been interpreted as evidence that the linear sequence of amino acid residues which comprises the covalent structure of the protein carries all the necessary information for directing the folding process, and it is just a short step to speculation that artificial synthesis or genetic engineering of novel sequences will lead to conformations with novel catalytic and control functions. But which sequences will lead to the desired conformation, and achieve it in reasonable time?

The latest contenders in pursuit of the answer are Ralston and De Coen (*J. molec. Biol.*, **83**, 393; 1974) and Nagano (*ibid.*, **84**, 337; 1974). Both follow current opinion in seeking the answer in nucleation, that is, in centres of conformational structuralisation which initiate and guide the subsequent folding up of the protein molecule.

Ralston and De Coen carry out conformational energy calculations on a variety of possible nucleating structures of varying complexity, though generally on sequence fragments of six or less residue units. Principally, the object of the exercise is to find the favoured (lowest energy) conformation for a variety of short sequences. In order to reduce the computation to reasonable proportions, some severe approximations are made. In particular, these workers exploit the Liquori concept (*Q. Rev. Biophys.*, **2**, 1; 1969) of a stereochemical alphabet in which only a small number of specified conformations is allowed for any amino acid unit and interactions between units merely determine which of these is the favoured one. Furthermore, not all sidechain conformations are adjusted in the search for the lowest energy confirmation, and neither are geometric variables other than rotations around single bonds. A true minimisation of the energy of the overall system could yield rather different results, and it is surprising that these workers do not apply established minimisation procedures even where these could be easily and fruitfully applied. Nevertheless, interesting conclusions are drawn concerning the favoured conformations of many short sequences which could, within much longer sequences, present the first appearance of structural organisation during the folding process and so act as nucleation sites.

It could be argued, however, that work of this kind is premature. Perhaps another, less ambitious phase should properly precede this one—the phase of consolidation of the methods used to calculate the conformational energies. Conclusions concerning nucleation are likely to be no more credible than the least reliable conformational energy function used. Complete substantiation of the results of Ralston and De Coen awaits full minimisation and the advent of more realistic conformational energy functions, or at least the substantiation of those already in use.

For this reason the calculations of Nagano would seem to be on safer ground. Nagano belongs to the school of investigators who predict nucleating structures not on the basis of conformational energy calculations but on the basis of statistical analysis of proteins of known sequence and conformation. A crude example of this type of approach might be the observation that glutamic acid tends to begin a run of residue units in an  $\alpha$ -helical conformation, and

therefore it might be predicted to do so in a protein of unknown conformation. The approach of Nagano, however, follows current trends in being much more sophisticated and quantitative, and takes account of the effect of pairs of residue units at different separations along the sequence. In his current paper, Nagano reports on the predictability of  $\alpha$  helix, extended chain regions which are potential candidates for  $\beta$ -pleated sheet structures, and looped conformations of the backbone, in proteins of recently determined conformation which do not appear in the data of the initial statistical analysis. How good are the predictions for new proteins of completely unknown conformation or when the conformation, if known, is not taken into account.

Nagano reports that the accuracy of the predictions “seems to depend very strongly on the actual data used”, and concludes that his predictions are less reliable for the new proteins. The predictions of  $\alpha$  helix are good, however, providing the protein is not rich in  $\beta$ -pleated sheet, and the predictions of  $\beta$ -pleated sheet are good if the protein is not rich in  $\alpha$  helix. The ‘goodness’ of a prediction is assessed in terms of a number of different accuracy statistics.

The results reported are at first glance disappointing to those who have learned from past publications in this field to expect steadily increasing degrees of accuracy. The important point is, however, that Nagano goes on to show that predictability can be enhanced by taking into account interactions between  $\alpha$  helix, potential  $\beta$ -pleated sheet and loops. In particular, the consequences of  $\beta$ -pleated sheet structures occurring in the vicinity of  $\alpha$  helices are considered. Nagano is therefore taking the first really serious look at the role in the folding of proteins of interactions between nucleating structures. Connoisseurs of the art of predicting protein conformation may feel that there is nothing entirely new in his reasoning, but at the very least Nagano presents a comprehensive preview of the problems to be encountered.

Nagano concludes his analysis of nucleation with a brief discussion of the final stage of protein folding after the topological pathway of the protein backbone in three-dimensional space is roughly determined. Recognising the importance of the close packing of the hydrophobic sidechains during this stage, he would do well to consider the recent papers of Richards (*J. molec. Biol.*, **82**, 1; 1974) and Wishnia and Lappi (*ibid.*, **82**, 77; 1974). Richards presents the most detailed analysis so far of the tight packing of atoms in proteins of known conformation, and he concludes that simple geometrical packing may provide useful criteria in guiding and evaluating trial structures in simulations of protein folding. Wishnia and Lappi, on the other hand, have investigated experimentally the binding of apolar ligands to hydrophobic sites, using the interaction of cyclohexane and n-heptane with  $\alpha$  and  $\beta$ -cyclodextrins as a model. If one considers the final stages of the folding of a protein as a folding of hydrophobic sidechains onto a previously established structure with a hydrophobic surface, then it is clear that work of this type will play an important part in providing thermodynamic parameters for the simulation of such a process.

What does seem clear is that the final stages of folding will be the most difficult to consider theoretically and therefore to predict. For simulating this stage of folding, the concepts and parameters obtained through investigations like those of Richards, Wishnia and Lappi will be necessary but not sufficient. The number of conformations to be treated and compared is clearly astronomical, and success will owe as much to the size and speed of future generations of computers as it does to the programs and data fed to them. But it is remarkable that one can now discuss the difficulty of predicting the final stages of folding, not the folding as a whole. This position has been achieved because of workers like Ralston, De Coen and Nagano who have done much towards reducing the number of initial and intermediate conformations in the folding process.

B.R.



## Towards improved ways of localising tumours

THERE are many current investigations aimed at improving methods for localising both primary and metastatic tumours. Non-specific radio-labelled proteins, for example, have been used by some investigators (Spar *et al.*, *Cancer*, **20**, 865, 1967; Bonte and Curry in *Radioactive Isotopes in the Localisation of Tumours*, 80, Heinemann, 1967). Recent findings in experimental and human tumour immunology have, however, generated renewed interest in the possible use of specific reagents, namely radiolabelled antibodies, as a novel type of localising agent.

The success of this approach will depend, among other considerations, on the specific activity of the labelled antibody, its specificity for the tumour cells and on tumour site, cell composition and size. Although little success has been claimed for such studies using some experimental tumour systems, specific and significant *in vivo* localisation of labelled antibodies against a tumour-associated transplantation antigen of a methylcholanthrene-induced rat tumour was attained by Izzo *et al.* (*Proc. Soc. exp. Biol. Med.*, **139**, 1185; 1972).

The carcinoembryonic antigen (CEA) is a human oncofetal antigen associated with the glycocalyx of tumour cells, and is released into the plasma and other body fluids. It was originally considered to be associated solely with endodermally-derived carcinomas (Gold and Freedman, *J. exp. Med.*, **122**, 467; 1965); further studies have shown that CEA, or 'CEA-like' materials, are released into the body fluids from a wide variety of neoplastic and some non-neoplastic conditions (see, for example, *Br. J. Cancer*, **26**, 335; 1972). Last year, Primus *et al.* (*Cancer Res.*, **33**, 2977; 1973) reported that a mucus-secreting carcinoma growing in immunologically-depressed hamsters and derived by serial propagation in hamsters from a human

colonic carcinoma, exhibited preferential uptake of radio-labelled heterospecific anti-CEA antibodies. In this week's issue of *Nature*, Mach and his colleagues now report similar findings for a human colonic carcinoma growing in nude mice and that this antibody accretion may be detected by suitable external scintillation scanning equipment. Unfortunately, in both studies, the labelled antibody was not located specifically in tumour tissues and there was a long delay after its administration before optimal tumour content of the label was attained.

Iodine is not the ideal scanning isotope; so far, it has only yielded labelled antibody with a relatively low specific activity. Isotopes with superior scanning properties such as technetium-99m may give better results, although the initial rate of isotope accumulation will be important in determining the usefulness of the method.

Other practical limitations include the presence of significant amounts of circulating antigens which may bind the heterospecific antibody and the presence of appropriate autoantibodies on the tumour cell surface. Both phenomena will serve to decrease the amount of labelled antibody eventually located in the tumours. The studies of Primus and Mach and their colleagues have been carried out in optimal conditions of tumour site and type, yet the smallest detectable lesion weighed 200 mg and was located superficially. Moreover, the presence of extensive tumour necrosis was also a limiting factor which, when considered with the problem of circulating antigen excess, reveals that there are many difficulties to be overcome before hepatic metastatic lesions of colorectal carcinomas with their characteristic central necrosis will be visualised in this manner in patients.

Consequently, this approach to tumour localisation does not have immediate clinical applicability. It does, however, reveal another possible area of fruitful investigation which may eventually yield not only improved diagnostic aids but also therapeutic regimes through the use of antibodies, coupled to anticancer agents.

A.M.N.

## Beyond systems ecology

WITH the increasing disturbance of natural systems by modification of the environment, the need for a way to predict the behaviour of complex ecosystems is rapidly becoming paramount. Because of the scale of the problem, the study of ecology is moving away from simplified systems and the analysis of elemental interactions towards systems in which the numbers and kinds of interactions are not under control and cannot be isolated. Extrapolation from two- or three-population models to large scale systems is often inappropriate and can give drastically misleading results.

The first attempts to derive a predictive theory of complex ecosystems were based on techniques well known to engineers under the general heading of systems analysis. A systems model is defined in terms of elements, each of which has one or more inputs and outputs and is specified in terms of the relationships between the various inputs and outputs of each element. With this very broad definition virtually all models are systems models of some kind but the term is usually used in cases where the number of elements in the system is large enough for detailed analysis of each one to be impossible.

Systems models have been applied on a small scale to ecological problems for the past one hundred years or so, usually in agricultural pest control or wildlife or resource management. The emphasis in most of the earlier applications was on the factors which affect a single population, usually one of commercial importance. These systems models were successful and widely used but limited

in extent by the amount of calculation necessary to analyse them. With the development of high-speed computers, Watt of the University of California, Davis and Holling of the University of British Columbia, and others, recognised that systems models in ecology could be used on a larger scale. The logical basis for their approach to the problem is the same as in the simplest models, but the availability of computers made it possible to use the models to determine and use the input-output relationships for all the populations within a community rather than only one of them.

The systems approach to ecology seemed so promising and the need for useful predictive models so great that between 1964 and 1972 funding of participation in the International Biological Program (IBP) has been at the level —unheard of in ecological research—of more than \$40,000,000 per year. Ecologists, engineers, meteorologists and mathematicians were gathered for the study of several systems ("biomes"), such as deciduous forest, intertidal zone, short and long grass savannah and desert. Extensive measurements were proposed as the basis for equally extensive computer models whose value could be determined only by a full scale program.

One of the reasons for the appeal of the systems approach, particularly to those not directly involved in ecological research, is its apparent objectivity. Ideally a systems model could be constructed by somebody with no prior knowledge or intuition of the system he is studying. The relationship between the elements would become clear

after a sufficient number of measurements. Since one of the difficulties in understanding the behaviour of ecosystems is ignorance of potentially important interactions, the systems approach seemed to eliminate any bias by design.

Unfortunately the promise of systems ecology has not been realised. The accumulation of accurate measurements and the construction of the first systems models lead not to useful predictive models of the various biomes but a recognition of the need for more measurements and more elaborate models. The successful systems models reduced the scope and analysed some of the subsystems in detail. Two problems have contributed to the difficulties encountered in the IBP studies. First, although computers are becoming larger and faster, there are still finite limits to the amount of information which can be analysed. The number of potential interactions increases with the square of the number of elements and the addition of one new element to the model requires the measurement of its relationship with all previous elements. Although common sense can reduce the number of interactions without reducing the objectivity of the approach (mice do not directly ingest soil arthropods), the quadratic increase remains. Second, the uncertainty and error inherent in ecological measurements greatly reduces the utility of a large model. A 5% error in each interaction might not significantly affect the predictions of a three-element model, but could invalidate those of a twenty- or thirty-element model.

An alternative to the systems approach has been developed independently by Levins of the University of Chicago and May of Princeton (*Proc. N.Y. Acad. Sci.*, in the press). "Loop analysis", as Levins calls it, is essentially hierarchical and depends on the initial assumption that the interaction between two elements of the system can be determined to be positive, negative or zero. It is thus formally equivalent to analysis of matrices with entries of +1, -1 or 0. In some simple cases, many of the important properties of a system can be determined without knowledge of the strengths of the interactions; the signs are sufficient. Levins (*Stability and Complexity in Model Ecosystems*, Princeton University Press, New Jersey, 1973), describes some examples for which the stability of the system and the sign of response of one of the elements to perturbations of one of the other elements can be determined.

The potential utility of loop analysis is not limited, however, to such simple systems. Although the results for more complex systems may be indeterminate when the behaviour depends on the strengths of the interactions, loop

analysis can identify those interactions or combinations of interactions (loops), which are the most important in determining the behaviour of the systems and thus provides the technique for reducing drastically the number of measurements which are necessary for a predictive model.

There are, of course, limitations to this approach. There is a possible bias introduced by the assumed signs of the interaction terms. There is no objectivity in the initial specification of the loop diagram, so that it is dependent on the intuition of the researcher. This means that it is necessary to investigate the consequences of ignoring certain interactions before reaching any conclusions. Further limitation is that if most of the interactions are in fact important in determining the behaviour of the system, nothing is gained over the systems approach. When a ecological system is inherently complex, loop analysis cannot simplify it, but when the system has had some simple aspects (and many ecological systems do if viewed correctly) then loop analysis can often reveal the simplicity.

From a Correspondent

## Narrowest room temperature Mössbauer resonance

from a Correspondent

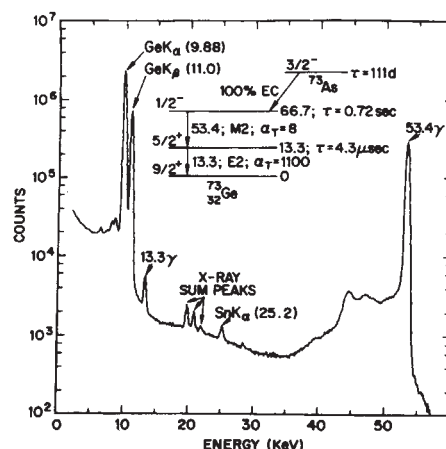
A RECENT paper by Raghavan and Pfeiffer (*Phys. Rev. Lett.*, **32**, 512; 1974) has shown that the Mössbauer effect can be studied using  $^{73}\text{Ge}$ . The 13.3-keV transition to the ground state has long been one of the most attractive candidates for high resolution experiments because of the extreme sharpness of the resonance ( $E\gamma/\Gamma_{\text{nat}}=10^{14}$  where  $E\gamma$  is the Mössbauer  $\gamma$  energy and  $\Gamma_{\text{nat}}$  is the theoretical value of the full line width at half maximum of the nuclear transition), and because the low energy of the  $\gamma$  photon assures that a high probability of recoil-less transitions will result even at room temperature. The observation in this paper that resonance does occur indicates that a potential 28-fold improvement in  $E\gamma/\Gamma_{\text{nat}}$  over the 14.4-keV transition in  $^{57}\text{Fe}$  is now available. This constitutes a major advance in the search for a high resolution tool for use in physics, chemistry and technology of germanium.

The width of the absorption line reported is about seven times  $\Gamma_{\text{nat}}$  but despite this broadening it is the narrowest Mössbauer line observed so far at room temperature. This extremely sharp line could, theoretically at least, measure very small changes in the s-electron density, the electric field

gradient and the magnetic field at the Ge nucleus.

The paper does indicate, however, the severe experimental difficulties involved in the use of this isotope as a source of extremely sharp electromagnetic radiation. In the figure, the  $^{73}\text{As} \rightarrow ^{73}\text{Ge}$  decay scheme, recently clarified by the use of high resolution Si(Li) detectors, is shown. The main problems which arise from this decay are, first, the high internal conversion coefficient,  $\alpha_{\text{IC}}$ , of the 13.3-keV  $\gamma$  photon of 1,100 (that is, only 1  $\gamma$  photon in 1,100 escapes from the  $^{73}\text{Ge}$  atom without being internally converted into an electron); second, the close proximity of the  $\gamma$ -photon energy to the K-absorption edge of Ge ( $=11.1\text{-keV}$ ); and, third, the low natural abundance of  $^{73}\text{Ge}$  in natural Ge of 7.6%. Because of the first two problems the resonance cross section is smaller than the nonresonant electronic absorption cross section in Ge at 13.3-keV ( $\sigma_{\text{res}}/\sigma_{\text{el}}=0.5$ ). This is an unusual and unwanted situation in a Mössbauer experiment. In practice, therefore, a choice has to be made between a thin absorber which reduces the potential of the resonant effect or a thicker absorber which will result in a reduction in the spectral quality of the detected photo peak at 13.3-keV. The use of enriched  $^{73}\text{Ge}$  absorbers are vital in this work.

Other difficulties arise from the extreme sharpness of the resonance. Narrow lines are only possible if the source and absorber nuclei are sited in environments as free as possible from all sources of inhomogeneous broadening. In the experiments reported, a fairly intense source of  $^{73}\text{As}$  embedded in a single crystal of high purity Ge and a single crystal absorber prepared with enriched  $^{73}\text{Ge}$  isotope were used. The velocity spectrometer employed a conventional electromechanical drive operating in the velocity range  $\pm 88 \mu\text{m s}^{-1}$ . This very small Doppler velocity, similar in magnitude to the velocity of the minute hand of a wrist



Energy spectrum of  $^{73}\text{As}$  source together with decay scheme of  $^{73}\text{As} \rightarrow ^{73}\text{Ge}$  (from Raghavan and Pfeiffer).



watch, is more than sufficient to scan the resonant line. This extremely small velocity range requires the apparatus to be isolated from building vibrations and the equipment was, therefore, set on a pneumatically suspended granite table.

Raghavan and Pfeiffer are optimistic, because of the importance of germanium technology and the extremely high purity and crystal perfection which can now be achieved by this technology, that the 13.3-keV transition of  $^{73}\text{Ge}$  will become a powerful new tool in high resolution Mössbauer spectroscopy. The experimental problems, however, are quite formidable. Since  $\alpha_T$  is so large, it would seem that a conversion electron detector which would detect the 1,100 internally converted electrons instead of, as in this paper, trying to detect the one resonant  $\gamma$  photon which escapes would reduce one of the experimental difficulties. Possibly one could use a  $^{73}\text{Ge}(\text{Li})$  detector as both absorber and detector. There is no doubt, however, that  $^{73}\text{Ge}$  Mössbauer spectroscopy does suggest many exciting possibilities to both the theoretician and experimentalist.

## Reindeer overgraze in South Georgia

from our Plant Ecology Correspondent

THE introduction of mammalian herbivores by man has resulted in a number of ecological problems in many parts of the world. In the British Isles the introduction of the rabbit, probably in the eleventh or twelfth centuries AD, imposed a new constraint upon the development of many vegetation types. The effects of these animals in modifying the composition of grassland swards was ably demonstrated on the South Downs by Tansley and Adamson (*J. Ecol.*, **13**, 177; 1925) who first used enclosure as a technique for this purpose. Subsequently much work has been done on this subject, particularly since the myxomatosis epidemic of 1954 (see, for example, Thomas, *J. Ecol.*, **48**, 287; 1960; White, *ibid.*, **49**, 113; 1961).

The reindeer is another mammalian herbivore which has been transported to new areas by man, though not as widely as in the case of the rabbit. In 1909 eleven animals were released on the Antarctic island of South Georgia and further introduction continued until 1925 in other parts of the island. The populations have remained isolated from one another, but by 1958 the original Barff Peninsula herd had risen from 11 to 4,000 animals. Numbers have increased even further in recent years since hunting ceased in 1964. There is evidence that sheer pressure of numbers is forcing an extension of



Reindeer grazing *Deschampsia antarctica* sward now invaded by *Rostkovia magellanica* in the Whale Valley, South Georgia. Photograph taken in January 1972 by P. Stone (from Lindsay, *Bull. Br. Antarct. Surv.*, No. 35; 1973).

range despite unfavourable environmental conditions such as steep mountain slopes and glaciers with abundant crevasses.

Lindsay (*Bull. Br. Antarct. Surv.*, No. 35, 101; 1973) has reviewed the effects which high reindeer stock densities may be having on the vegetation of South Georgia. Unfortunately, past documentation of the flora has been largely non-quantitative and this makes it difficult to be sure that vegetation is indeed changing. To supplement this anecdotal evidence concerning the past flora of the island, Lindsay makes a comparison of vegetation in grazed areas with that in similar areas which as yet have no reindeer. This method of approach also has certain disadvantages, for one can never be sure that all other factors are equal in the two sites.

Despite these difficulties, Lindsay does present evidence which suggests that the intensive grazing of reindeer is severely modifying the composition of the vegetation. Some plants, such as *Acaena adscendens* are little changed in overall frequency, but are reduced to a creeping, rhizomatous growth with few leafy shoots. This species was recorded in 1890 as being "richly developed" in the area. In areas of very high stock density, such as the Barff Peninsula, *Acaena* is almost completely eradicated; evidently the constant removal of leafy growth can weaken the species over long periods. Some species such as the grass *Festuca erecta* and the rush *Rostkovia magellanica* are apparently unpalatable and hence have increased in abundance as a result of the reindeers' selective grazing. *Festuca*, however, does seem to be sensitive to heavy trampling.

The most serious effects of grazing and trampling are upon the lichens. Many species abundant in the ungrazed areas are now almost or completely absent from the grazed regions. The

two most abundant lichens affected are *Cladonia rangiferina* ("reindeer moss") and *C. furcata*. The former has vanished completely and the latter is reduced in cover abundance by a factor of ten as a result of the heavy grazing pressures. Experimental work on reindeer in the Arctic (for example, Pagau, *J. Range Mgmt.*, **23** (2), 95; 1970) has shown a similar demise of lichens under heavy grazing and trampling pressures. Such an effect is not of widespread importance in most Arctic areas because the density of reindeer is normally far lower than in South Georgia. For example, caribou in Newfoundland are estimated to have a density of one animal per 110 km<sup>2</sup>; densities in South Georgia may locally rise as high as one animal per 0.07 km<sup>2</sup>.

The very high densities of reindeer attained in this new environment free from predation is adequate to explain the gross vegetational changes suggested by Lindsay's study. Two lessons should be learned from this work; in the first place the population levels of an introduced animal world should be strictly monitored and controlled and in the second place a more rigorous effort should be made to document the flora and fauna of areas in which such introductions are planned. Only in this way can the native biota be used as a clinical thermometer attesting the health or otherwise of a virgin community placed under an alien stress.

## Questioning isostasy

from our Geomagnetism Correspondent

THE observations made by the members of Pierre Bouguer's expedition to Peru between 1735 and 1745 were to lead to the discovery of one of the most important principles in geology. Bou-

guer's principal aim was to measure an arc of meridian, a procedure requiring repeated determinations of the vertical—and he was fully prepared for the local errors arising from the horizontal attraction of the Andes on his plumb-line. But to his surprise he found that the gravitational pull exerted by the Andes was "much smaller than that to be expected from the mass represented by these mountains!" More than a century later, after Sir George Everest had found similar discrepancies in the vicinity of the Himalayas, Airy (*Phil. Trans. R. Soc.*, **145**, 101; 1855) was able to use his "hypothesis of crustal balance" to explain this curious lack of mountain power in terms of an underlying mass deficiency roughly equal to surface load represented by the mountains themselves. Thus was born the principle of isostasy, although the operative word was not coined until more than 30 years later by Dutton (*Bull. phil. Soc. Wash.*, **11**, 51; 1889).

Today, few people would dispute that at equilibrium there exists in the Earth a certain 'level of compensation' above which all columns of material having the same cross-sectional area have the same mass. Moreover, even the old conflict between Pratt's (*Phil. Trans. R. Soc.*, **145**, 53; 1855) suggestion that isostatic adjustment takes place by lateral density variation above a level of compensation lying at constant depth, and Airy's view that the material above the level of compensation comprises a lower density crust overlying a higher density substratum, has been settled to most people's satisfaction by acknowledging that either or both mechanisms may apply in certain circumstances. The basic principle of isostasy is now so ingrained in the fabric of geology that few earth scientists would question that the crust in a region thrown out of isostatic equilibrium by an 'external' force such as glaciation will return to such equilibrium once the force is removed.

But Artyushkov (*J. geophys. Res.*, **79**, 741; 1974) now questions precisely that, arguing that "the isostatic state is generally unstable and differs from the stable position of the crust, which actually exists in many regions". What apparently persuaded Artyushkov to carry out the analysis leading to this remarkable conclusion was the acknowledged fact that, for no immediately obvious reason, many young orogenic regions (the Eurasian Alpine geosyncline zone, for example) are associated with strong isostatic anomalies, even though gravimetric data show the Earth's crust as a whole to be generally close to isostatic equilibrium.

In developing this problem point, Artyushkov notes that in a region whose horizontal scale is several times greater than the lithospheric thickness

the crust will recover equilibrium quite rapidly following the removal of external stress. For a region several hundred kilometres across, for example, the characteristic recovery time following glacial retreat is only of the order of thousands of years. But in young orogenic regions the crust is hotter than normal and the lithosphere is thinner, implying an even more rapid and complete restoration of equilibrium. In other words, the period of crustal recovery in such regions is much less than the typical duration of tectonic activity ( $10^5$ – $10^7$  yr), suggesting that on a regional scale the crust should be close to its equilibrium position.

One way of reconciling this supposed equilibrium with the obvious lack of isostasy would be to invoke viscous forces acting on the lithosphere from the asthenosphere, but the creation of the necessary viscous stresses would require improbably high asthenospheric flow rates of greater than  $10\text{ m yr}^{-1}$ . Instead, Artyushkov sets out to show that it is a mistake to assume that the equilibrium position of the crust is generally the same as the state of isostatic equilibrium; the two states are physically quite distinct and coincide only in special circumstances.

The core of Artyushkov's case is an extended mathematical treatment of an admittedly simplified model of the Earth's crust floating on the mantle. But it is sufficient to show that large non-hydrostatic stresses exist in the crust and lithosphere because of horizontal variations in crustal and lithospheric thickness and that these stresses are non-uniformly distributed throughout the depth. One consequence is that the crustal/lithospheric layer has two characteristic relaxation times, the first ( $T_1$ ) being associated with the vertical adjustment of the layer and the second ( $T_2$ ) with the horizontal spreading of inhomogeneities in the layer thickness.  $T_1$ , which is much smaller than  $T_2$ , is the time associated with the reaching of the 'equilibrium position' (that is, the mechanical equilibrium position) and this state only coincides with isostasy in the special case of a region where the crustal and lithospheric thicknesses are constant; conversely, unless the thicknesses are constant the state of isostatic equilibrium is mechanically unstable.

Because data on the vertical distribution of the stresses in the lithosphere are sparse, it is not possible to compare gravity anomalies predicted by the Artyushkov model with observed anomalies in any great detail, although there is general agreement between the two. Thus the model predicts very low gravity anomalies for platform lowlands located far from mountain regions, negative anomalies of a few

tens of mgal over ocean basins a few thousand metres in depth, and positive anomalies of a few tens of mgal over young orogenic regions—to take just a few examples. Practical demonstrations apart, however, the more important general point is that Artyushkov's analysis offers an explanation, in principle, of certain isostatic anomalies whose existence geologists and geophysicists have very often tended to gloss over. By the same token, it also seems to hit at one of earth science's most cherished principles, suggesting that even the most basic and time-honoured assumptions may benefit from critical re-examination.

## Sticks and carrots

from our Materials Science Correspondent

At a conference on the conservation of materials, sponsored by the Institution of Chemical Engineers and the British National Committee on Materials, on March 26–27 at Harwell, the emphasis was partly on primary metal supplies and the associated topic of substitution, with a low in the direction of plastics. Much of the conference, however, was targeted on the problems, both technical and political, associated with recycling. The political interest in this field was signalled by the opening address delivered by the Chief Scientific Adviser to the Cabinet, Sir Alan Cottrell, and of the Liberal peer, Lord Avebury.

What was for one observer the most interesting issue discussed at the conference was raised by P. F. Chapman (Open University)—a most cogent and persuasive speaker. His primary concern is with the energy content of primary metals implied by the energy input of the extraction and purification procedures. When this aspect is pushed into the forefront, then the systematic recycling of 'energy intensive' metals becomes particularly attractive. The problem is, how are the public and manufacturers to be persuaded to take the necessary steps—domestic conscientiousness and apposite engineering design—to increase recycling substantially?

Chapman quoted the parable of the common. A piece of common land can support twenty-five cows, and five smallholders each have the right to graze five cows there. All is well until one of them adds a sixth cow, to increase his income a little. All twenty-six cows go a little short of grass, including the rogue smallholder's sixth cow, but overall he gains; most of the loss, without any compensating gain, falls on the other four smallholders. In turn, they add cows, until in the end all are worse



off, and the last state is worse than the first for all. This might be a parable for the evils of inflation, but in the present context the point is that the incentive for self restraint is merely collective, whereas each individual in isolation has a financial motive for selfishness.

A case in point is the design of beer cans. Many of these are made of steel, with a top made of aluminium for its easier tearing qualities. Such cans are almost useless for recycling, since aluminium is an unacceptable contaminant of scrap steel. The manufacturer has an incentive to design a can in such a way that the consumer is most likely to buy it (I take it as axiomatic that most canned beer is of indistinguishable and mediocre quality!). The scrap merchant or the British Steel Corporation gains from a prevalence of aluminium-free cans, whereas the beer drinker and the can maker do not, and may even suffer inconvenience.

This last point, the fact that the financial benefit of improvements in the recycling technique does not go to the people who need to modify their behaviour to bring the benefits about, was also made forcefully by J. B. J. Williamson (Guest, Keen and Nettlefolds Ltd). He worded it differently: in the course of materials recycling, ownership of raw material changes from manufacturing industry to the user, until it becomes waste and belongs to everybody and nobody. This is why recycling in the manufacturer's plan ('prompt recyc-

ling') is so much more effectual than 'post-consumer recycling'. A recycling industry is defenceless against the vagaries of the public, and is so vulnerable to a fierce price-war with primary producers that it would have to concentrate totally and would have no scope for diversification—hence would be unattractive for investment. This difficulty is enhanced by the fact that technological improvements by users reduce demand for metals in short supply; thus a new, effective method for joining aluminium cable ends reduced demand for copper cables.

Yet an autonomous recycling industry is bound to come. Williamson was persuasive in arguing that the economic forces of the market will not favour recycling; but recycling will become much more attractive if (1) foreign primary sources become less reliable (he had disruption in mind rather than nationalisation) and (2) governments convert environmental priorities into fiscal incentives and threats.

This will not be easy—Chapman suggested that the building of aluminium smelters in Britain with government backing and incentives led to a reduction of aluminium recycling rather than a reduction in primary imports—but it is essential. Plainly manufacturers and consumers alike must either be cajoled with allowances and other financial incentives (for instance, to use all-steel or all-aluminium cans, and then to collect them after use) or they must be threatened with penalties if they do not

behave. The trouble with the stick is that civil servants have to think up a complete programme of legislation and the police have to enforce it. Yet such a programme can only be based on present technology: as Marx commented, the problem is not to understand the world but to change it, and the advantage of using the carrot instead of the stick—incentives rather than threats—is that industry would have a motive for developing new recycling processes and new manufacturing designs congenial to recyclers. There must be a mixture—but let there be incentives for carrot growers.

Sir Alan Cottrell remarked that the British Government will usually act if they have clear, unambiguous information. It is to be hoped that the Civil Service will provide that information and that legislation will follow.

## Dynamics of biomembranes

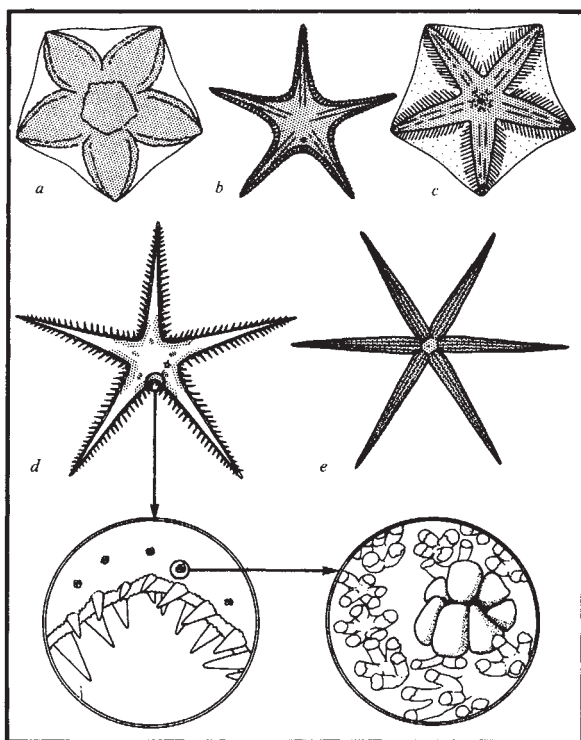
from a Correspondent

At an international conference in Titisee on March 21-23 on the dynamics of biomembranes, the proceedings began with a reminder by H. P. Zingsheim (Max-Planck Institute, Göttingen) of the difficulties of attempting to draw conclusions on the molecular structure of membranes from either fixed and stained sections or from freeze fractured preparations. Chemical studies by M. S. Bretscher (University of Cambridge) and investigations with purified phospholipases by R. F. A. Zwaal (University of Bern) which have revealed an asymmetric distribution of phospholipids in red cell membranes were discussed. There is, however, no evidence for similar asymmetry in other cell types: the biosynthetic origin of the asymmetry is also obscure. In connection with lipid phase transitions, D. Chapman (University of Sheffield) reported that transition temperatures can be altered by drugs, especially antidepressants, and H. Träuble (Max-Planck Institute, Göttingen) indicated that lung alveoli may utilise the phase transition properties of surfactant phospholipids during expansion and contraction.

Interesting points were made by E. H. Eylar (New Jersey) on two autoimmune diseases induced by injection of membrane proteins that may normally not be exposed *in vivo* to the immune system, namely, the A1 basic protein of the myelin of the central nervous system and sperm proteins: the sperm proteins have a possible immunological application in the control of fertility. Complex formation of the A1 basic protein with lipids in surface monolayers was dis-

## Luminescent echinoderms

Like many other marine animals, some echinoderms are bioluminescent. Herring (J. Zool., 172, 401-418; 1974) has found luminescence in two species of ophiuroids, six asteroids, nine holothuroids and two crinoids. This figure shows the main luminescent areas (stippled) of five asteroids: a, *Cryptasterias personatus*; b, *Plutonaster notatus*; c, *Hymenaster* sp.; d, *Pectinaster forcipulatus*; and e, *Hydrasterias ophidion*. The dorsal surface of *P. forcipulatus* bears fasciculate pedicellariae which do not occur elsewhere on the body but it is not known if they are involved in the luminescent response.



cussed by A. A. Demel (University of Utrecht) who reported that the amino terminal region of the protein seems to interact preferentially with lipids as this region is resistant to trypsin added to the subphase.

On the second day J. A. Lucy (Royal Free Hospital School of Medicine, London) drew attention to the possible role of increased lipid fluidity in membrane fusion. C. A. Pasternak (University of Oxford) then described experiments on membrane leakiness (not lysis) which may be an initial stage in virus-induced cell fusion; he also ventured the opinion that enzymes are not involved in this action of viruses. K. Simons (University of Helsinki) presented findings on the membrane glycoproteins of Semliki forest virus which resemble glycophorin in projecting right through the membrane. Considerable discussion was aroused by his intriguing proposal that release of virus by budding through the plasma membrane may involve recognition and interaction of nucleocapsid proteins with the cytoplasmically-exposed segments of these glycoproteins in the membranes of infected cells.

S. C. Kinsky (Washington University, St Louis) reported important studies on liposomes which he had rendered immunogenic by incorporating DNP-coupled, N-substituted derivatives of phosphatidylethanolamine. Appropriately substituted phospholipids thus seem potentially valuable in immunological studies on haptens: they may also be useful in the radioimmunoassay of drugs. Another investigation on liposomes was described by B. E. Ryman (Charing Cross Hospital Medical School, London) who has encapsulated enzymes within liposomes (of potential use in inborn errors of metabolism), and who is applying encapsulation to drugs, for example, 5-flourouracil, as this may permit the tissue specific application of relatively small doses of drugs.

Lysolecithin was a recurring topic, and there were discussions of whether or not small quantities might be involved in virus-induced cell fusion, of its acylation with unsaturated fatty acids in thymus cells stimulated by concanavalin A, its possible role in cellular junctions and its facilitation of concanavalin A-induced agglutination in chicken erythrocytes during which H. Fischer and his colleagues (Max-Planck Institute, Freiburg) made several contributions.

A lively exchange centred around the relevance of Mitchell's hypothesis in the transport of galactosides, amino acids and other molecules across membrane vesicles prepared from *Escherichia coli* that is coupled to the oxidation of D-lactate (H. R. Kaback, Roche, New Jersey; W. R. Konings, Haren, Nether-

lands). Finally the mode of action of antibiotics on membranes, which seems still to be a very open question, was pursued by B. Deuticke (Technical High School, Aachen) who concluded that the presence of proteins scarcely modifies the action of these drugs on the lipid components of membranes, and by B. de Kruijff (University of Utrecht) who discussed the formation of some interesting, albeit speculative, molecular complexes of polyenes with cholesterol in relation to their biological activity.

The meeting was sponsored by Dr Karl C. Thomae, Biberach/Riss, subsidiary to C. H. Boehringer, Ingelheim and was organised by L. L. van Deenen (University of Utrecht) with H. Schroeder (Thomae).

## Is the bass on the decline?

from our  
*Marine Vertebrate Correspondent*

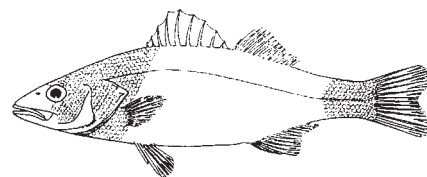
THE bass (*Dicentrarchus labrax*) is a well known species to the sea angler; it is highly rated as a fighting fish, it grows to a fair weight, and has a relatively palatable flesh. In British waters it is widely distributed, but common only in the south and south-west, although it occurs as far north as Scotland where especially in the north-east it is rare. Until recently, however, its biology was virtually unknown; there were no data on its ageing and growth, little was known about its food, and the only knowledge of its spawning habits and early life was derived from elementary observations. The value of the bass to anglers first prompted research by staff of the Inland Fisheries Trust, Dublin who contributed to knowledge of the bass's biology in Irish waters (Kennedy and Fitzmaurice, *J. mar. biol. Ass. U.K.*, **52**, 557-597; 1972). Knowledge of this fish has now been extended in a report by Holden and Williams on dynamics of bass in English waters (*J. mar. biol. Ass. U.K.*, **54**, 91-107; 1974).

Holden and Williams's study was initiated by the concern expressed by angling bodies at the apparent decline in bass populations in English waters. A programme of tagging was begun with the aid of members of the National Federation of Sea Anglers, in which anglers tagged 954 bass in 1970 and 1971 mainly on the coast of Devon. Of these fish, 59 were recaptured, most (45) within 16 km of their release point and the majority (44) within the year of tagging. The exceptions, however, show that there were long-range movements, some of them over an extended period, the longest being 1,066 days at liberty. Most of the fish represented by returns had evidently made coastwise migrations, although one fish tagged on

the South Devon coast was captured after 222 days in mid-Channel to the west of the Channel Isles.

On the basis of these tagging returns the authors suggest that the life history of bass in English waters falls into two phases. The first is the nursery stage when young fish of about 10 cm length enter estuaries in which they stay for 1 or 2 years, leaving usually as 3-year-old fish. The second phase is one of greater mobility when, as adult fish, they migrate coastwise and even offshore, possibly seasonally.

This synthesis of the first years agrees with the results of Kennedy and Fitzmaurice who also found young bass to live in estuaries, creeks and lagoons, but concluded that mature bass do not make extensive migrations. The different conclusions of Holden and Williams may be due to the fact that the Irish tagging was confined to the southern coasts of Ireland where bass are abundant and present all year round. A substantial part of the tagging effort of Holden and Williams was expended in similar areas of south-west England, however, and the data in confirmation of their theory amount to only 12 returned tags, of which only 6 were of distant migrations. In support of this rather slender data they cite communicated information from another source of three bass tagged off Anglesey which were recovered in Cornwall and Devon, but of this critical evidence no further information is advanced.



*Dicentrarchus labrax.*

The apparent contradiction between the conclusions of the two groups is important. If Kennedy and Fitzmaurice are correct in assuming that the bass populations are mainly sedentary, then heavy fishing could quickly lead to severe local depletion of the stock. Conversely, if Holden and Williams's conclusions are accepted the stock of bass is at least national, and heavier exploitation can be permitted locally without undue damage. It is unfortunate that in this critical area there is conflict of opinion and little data from which to draw conclusions.

With regard to the alleged decline in bass fishing, both pairs of authors consider the cause to be the failure of successive year-classes caused ultimately by climatic changes. Holden and Williams even venture to predict that if present climatic trends continue, bass could possibly become a rare species even in southern British waters.



# Fortification of foods with amino acids

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*In this article Dr Altschul discusses how the addition of specific amino acids can solve some nutritional problems, chiefly by reducing the pressure on protein produced from the land.*

FORTIFICATION (or supplementation) of a food with amino acids is a process whereby the limiting deficiency of amino acids in a diet is eliminated or reduced by adding small amounts of one or a small number of amino acids. As such it becomes a practical tool for selectively improving the protein component of a diet without requiring the addition of other amino acids or food components already present in adequate amounts. Usually, the deficiencies of one protein source are balanced, in part, by mixing with one or more other protein sources which contain an excess of the amino acid deficient in the first: corn plus beans, or a cheese and bread sandwich, for example. The result is the creation of a new mixture of better protein value than the first. The use of specific amino acids allows adjustment of the amino acid composition in the protein to the exactly desired balance, that is in balance with the second limiting amino acid. Such adjustment introduces another option for increasing protein supply at low cost.

Supplementation with amino acids is normal practice in formulating animal feeds<sup>1</sup>. For example, corn and soybeans are the major energy and protein ingredients in nonruminant feedstuffs in the United States. When soy represents a substantial portion of the protein in the mixture with corn, then methionine is limiting; addition of DL-methionine improves the protein quality of that mixture. When soy provides a small proportion of the protein in the diet, the mixed feed does not provide enough lysine to balance the deficiency of lysine in the corn. In such circumstances addition of lysine is required for optimum nutrition. This can be obtained from fish meal or by addition of the amino acid itself.

Addition of amino acids also can be part of a system of improving the quality of human diets. Addition of methionine makes certain legume foods better sources of protein. It has been suggested that the addition of less than 1% of methionine to manioc flour would improve the value of cassava bean diets in Brazil by improving the protein quality of the beans<sup>2</sup>. Bread supplemented with lysine is a far superior source of protein than ordinary bread. Fortification may be a means of reducing the cost and improving the delivery of protein in institutional meals and could also improve the special delivery systems organised for infant and maternal feeding or for feeding of the aged.

Hegsted<sup>3</sup> estimated that white flour containing 3.2% utilisable protein will have its protein impact increased to 5.3% on addition of 0.2% L-lysine·HCl. On this basis it can be estimated that the one million tons of wheat consumed in Tunisia annually would gain the equivalent of 20,000 tons of utilisable protein by addition of lysine. Relief shipments of grain to avoid famine can be made more complete sources of nutrients, particularly protein, by fortification either at the point of shipment or at destination.

Nutrition is not merely calorie or protein nutrition; it is meeting the total nutrient requirements of the body. Thus, mechanisms which allow addition of amino acids to

single foods or diets can also be the means of adding other micronutrients such as vitamins and minerals.

The literature on amino acid fortification has been reviewed extensively<sup>4-7</sup>. Eight essential amino acids—leucine, isoleucine, lysine, methionine, valine, threonine, phenylalanine and tryptophan—are required by human adults; histidine is required in addition by infants. The highest requirement for the infants is leucine and the lowest is tryptophan. Adults require less protein and less of the essential amino acids—19% as essential compared with 40% for the infant—and the spread in requirements among the essential amino acids is also less than for the infants. Radical departure from the optimum pattern can cause amino acid imbalance with many consequences including reduced growth<sup>8,9</sup>. This can result from excessive addition of a single amino acid to a diet or from complete reliance on single sources of protein such as corn<sup>10</sup>. Thus, any supplementation with amino acids must be controlled to avoid such imbalance.

Amino acids rarely occur free in nature; they are supplied as proteins in food. Essential amino acids and the nitrogen required for growth and maintenance of body tissue nitrogen are thus supplied by the food protein. Amino acids may also be supplied as protein hydrolysates or as mixtures of free amino acids, the latter either orally or parenterally. The metabolism of amino acids presented as proteins differs somewhat from that of free amino acids, particularly in the treatment of temporary excesses, but all the evidence supports the idea that for practical purposes amino acids in proteins and free amino acids can be considered as interchangeable<sup>11</sup>.

Certainly there is every evidence that a food protein supplemented properly with amino acids will have the expected improved impact. Many generations of rats have been raised on totally synthetic diets in which proteins were completely replaced with specific amino acids and other sources of nitrogen<sup>12</sup>. Humans have been kept in nitrogen balance by completely synthetic diets consisting entirely of the essential amino acids and other nitrogen sources<sup>13</sup>. Infants grow normally on wheat protein plus lysine as the sole source of protein<sup>14</sup>. However it is measured, the fact emerges that plant sources of protein properly supplemented with amino acids are adequate to support growth in animals and man.

## Amino acid supplementation in practice

By far the greatest utilisation of pure essential amino acids has been in animal feeds; the most used has been methionine. The United States consumes about 22,000 tons of DL-methionine annually; world capacity is 92,000 tons<sup>15</sup>. Prices for DL-methionine are in the range of \$1.20 to \$1.60 per pound. Recently, as the price of soy has increased, formulas are being reconsidered to use the least amount of soy protein necessary to complement the amino acids of corn. For many feeding purposes the protein content of the diet can be relatively low; the soy then is required primarily to furnish tryptophan; the deficit of lysine can be overcome by providing additional L-lysine. Consumption of lysine in the United States is at the level of 6,000 tons annually at a price of \$1.00 to \$1.60 per pound.

TABLE 1 Estimates of cost of fortifying cereals with amino acids

Case 1: Rice<sup>29</sup>

| Ingredients                                         | Cost Assumptions    | Cost Estimate        |
|-----------------------------------------------------|---------------------|----------------------|
| Rice (milled)                                       | \$200 per 1,000 kg  |                      |
| L-Lysine HCl (0.15%)                                | \$1.80 per kg       | \$13.60 per 1,000 kg |
|                                                     |                     | or                   |
| L-Threonine (0.05%)                                 | \$19.40 per kg      | 6.8% of cost of rice |
| Fortification granules and cost of addition to rice | \$1.20 per 1,000 kg |                      |

## Case 2: Wheat\*

|                                                                         |                       |                            |
|-------------------------------------------------------------------------|-----------------------|----------------------------|
| Wheat                                                                   | \$200 per ton         |                            |
| L-Lysine HCl (0.23%)                                                    | \$10 per ton of wheat |                            |
| Vitamin A (250 million IU g <sup>-1</sup> ) (0.033 g kg <sup>-1</sup> ) |                       | \$12 per ton. <sup>†</sup> |
|                                                                         |                       | or                         |
| Niacin (0.019 g kg <sup>-1</sup> )                                      | \$2 per ton of wheat  | 6% of cost of wheat        |
| Riboflavin (0.0025 g kg <sup>-1</sup> )                                 |                       |                            |
| Thiamin (0.0016 g kg <sup>-1</sup> )                                    |                       |                            |
| Iron (0.055 g kg <sup>-1</sup> )                                        |                       |                            |

\* P. R. Crowley, personal communication.

<sup>†</sup> Cost of ingredients includes cost of preparing and adding a premix.

Methionine has been added at 0.5% of soy protein in infant formulas; such formulas have been judged equivalent in protein value to the proteins of meat, eggs, and milk<sup>16</sup>. Interest in applying lysine fortification to human foods is relatively recent and coincided with the development of methods for the production of L-lysine at practical costs. It was recognised early that lysine supplementation offered an unprecedented opportunity to improve protein nutrition, particularly for those who could not afford adequate protein and who depended heavily on cereals for most of their calories and proteins<sup>17-19</sup>.

The applications are few however. Some cold breakfast cereals and breads are fortified with lysine in the United States. There are instances of rice and noodle products fortified with lysine in Japan. Certain Incaparina formulas are fortified with lysine<sup>20</sup>. Interest in amino acid fortification of human foods is certainly on the increase but at the time of writing, the utilisation of this option is at too low a level to make a significant contribution to human protein supply.

The economics of amino acid fortification of human foods are complex. The population is heterogeneous compared with the homogeneous animal populations; different human age groups differ in their requirements of protein and lysine. Moreover, not all diets are always deficient in protein. The poor, and particularly the vulnerable among them, are most likely to be deficient in protein. Amino acids improve protein quality, they add no calories to the diet; thus, it is difficult to compare in a general way costs and benefits of this approach to nutrition compared with other options. The comparison must be on specific approaches to well-defined groups.

### Issues surrounding amino acid fortification

The main issue is how to use amino acid fortification to advantage to provide more protein at a lesser cost to people who do not get sufficient. Since this will involve a government decision to allocate scarce resources or to modify regulations involving addition of amino acids to the food supply, the ease and rapidity with which such a positive decision is made depends in part on the nature of the consensus among scientists who advise government. The following are some of the issues being discussed within various countries and in United Nations' agencies regarding amino acid supplementation of foods.

**Calories or protein** This involves amino acids only peripherally. The question is whether the most important deficiency in the diets of poor nations is protein or calories.

Amino acid addition represents the extreme of adding protein under circumstances where no additional calories are provided.

Concern for the protein aspect of malnutrition was intensified by the discovery of the aetiology of kwashiorkor, recognition of its wide distribution in underdeveloped countries, and the realisation that clinical protein-calorie malnutrition is only a small aspect of a more general syndrome involving specific protein deficiency<sup>21</sup>. More recently, important members of the nutrition community have insisted that the major problem in poor countries is lack of calories, that the protein quality is adequate, and that what is needed is more of the same kind of food<sup>22</sup>. Latest revisions of standards of protein requirements for normal healthy humans are lower than before<sup>23,24</sup>. Although there are many uncertainties, such as the increased needs of the sick and the relationship between minimal and optimal protein nutrition, the tendency is to translate such nutritional judgements into recommendations for public policy.

The meaning of 'more of the same kind of food' needs more inquiry. The proven existence, or lack, of protein malnutrition is just one part of the issue. The major problem is the interdependence of calorie and protein supply: there is a protein-calorie tradeoff. This occurs because calories are cheaper than protein. Poverty forces a reduction in the amount of protein food consumed (legumes or animal products) in favour of the cheaper foods (cereals, root starches and sugar).

Stress for more calories challenges agricultural capacity to maintain quantity and quality of protein supply. Increased population demands an increased food supply merely to maintain nutritional conditions at the existing level. But there is no unlimited ability to expand food production. The great increase in grain production—the "Green Revolution"—which has unquestionably had a great impact on food availability has come about because high yield varieties of wheat and rice were planted. These required more fertiliser and energy input and more irrigation. More acreage was put into grain production at the expense, in some instances, of that devoted to legume production.

In India, for example in the sixties, *per capita* calorie availability fluctuated around 2,050 kilocalories: first dropping to 1,950, rising to 2,200, and then returning to 1973 estimates of 2,050. Protein availability remained about the same in that decade at about 53–55 g *per capita* per day. This is a great achievement considering the population increase during this period, but percentage of protein from pulses (legumes) decreased from 23% in 1959–61 to 15% in 1973. Without a compensatory increase in animal protein, this trend represents a serious threat to protein balance, particularly to the lysine content of the diet<sup>25</sup>. An FAO report of projections of percentage of calories as protein in South Asia lists this value as decreasing from the present 10.2% to 9.8% in 1980 (ref. 26). As the percentage of protein calories is reduced, more reliance is placed on cereal protein and less on concentrated protein sources such as legumes and animal proteins, which also have an excess of lysine. Therefore, not only is the percentage of protein as part of the calories reduced but also the quality of the protein. The reduction is far more serious than the numbers would indicate.

Added to this problem is the increasing demand for protein by nations whose income *per capita* is increasing. Those that have had high incomes for some time have enjoyed a high protein intake gained at the expense of huge excesses of cereals to feed the animal population. In the United States approximately one ton of cereal is available *per capita* but only about 150 pounds are eaten each year by humans directly; the rest is fed to the animals. The grain equivalent calories required *per capita* per day in the developed countries can be estimated as 8,000 kilocalories



compared with 3,000 for the underdeveloped countries<sup>27,28</sup>. As their income increases, other countries are trying to catch up, as evidenced by the increased sales of cereal grain and protein concentrate to Russia and Japan, as well as Western Europe. Thus, there is the situation where, almost explosively, the world has been forced to recognise a worldwide shortage of protein or the ingredients with which to produce it. The United States had a temporary embargo on the export of protein-rich commodities and feedstuffs in the summer of 1973.

In the face of such developments it is inconceivable that any country or any group can say that there is no protein problem. Certainly, planning on that basis courts disaster.

**Natural or synthetic sources of amino acids** There is a preference to obtain the deficient amino acids by breeding them into the plant rather than by supplementation. This idea has attractive features. It departs least from the norm: the corn, wheat or rice is almost the same, but has more protein and lysine. If this could be achieved finally without any reduction in yield for the particular crop, then this is indeed the best idea.

The cereal most advanced in this direction is corn. High lysine corn has made considerable progress<sup>29</sup>. Nevertheless, the new corn has not been planted in a practical sense any more than amino acid fortification has been instituted, because in most instances the yield is less, there are some problems of acceptance of conventional food products derived from these varieties, and there is need for social organization to ensure that the correct seeds are planted and sold. Whether or not it will ultimately be possible to develop the improved corn so that it will yield and maintain increase in yield as well as the ordinary corn remains an open question. At present yield per acre is all-important. A more likely outcome could be a combination of breeding for improved quality with fortification to complete the process.

**Unfamiliarity with the concept** Supplementation with amino acids or vitamins and minerals is an unfamiliar concept in most places. To increase familiarity and provide a means for seeing what could happen in reality, three field trials have been organised by the Agency for International Development (US) in cooperation with the United States Department of Agriculture, several governments and their scientists, academic institutions, and some industrial firms providing ingredients and technology<sup>30,31</sup>. These involve addition of lysine and vitamins to wheat flour in Tunisia; fortification of rice with vitamins, lysine, and threonine in Thailand by mixing synthetic granules with natural rice in the village mill<sup>32</sup>; and fortification of corn at the village mill with small quantities of soy flour plus lysine in Guatemala.

In all instances baseline data are being obtained on the populations, particularly the children, and information is being collected on the cost of fortification, its effect on the amino acid composition of the food supply, and on whatever changes can be determined in health status that might be attributed to improvement in the protein value of the foods. It is realised, of course, that measurement of changes in an entire population and the determination of the important cause and effect relationships is difficult, if not impossible. Certainly, that fortification with amino acid improves the protein value of a cereal is beyond doubt. The issue is the degree to which the effect of nutrition intervention alone on public health can be determined.

**Cost** Since the object of amino acid fortification is to reduce the cost of protein for those who can least afford it, government subsidies are involved, so the cost of amino acids and lysine, in particular, is critical. Sample calculations of the cost of fortification of wheat and rice are given in Table 1.

Slessor<sup>33</sup>, in his discussion of energy subsidy in food production, considered the case of fortification of 'Food for Peace' shipments of wheat from the United States in 1966. To have fortified  $30 \times 10^9$  pounds of wheat with  $32 \times 10^6$

pounds of lysine would have required an energy subsidy of  $390 \times 10^9$  kilocalories, and this would have provided a nutritionally feasible diet for one hundred million people. He also said that the same energy subsidy expended in the importing country on marginal grassland could produce 38 pints of milk per person. Extrapolation of the calculation derived earlier<sup>3</sup> to this case shows that the fortification route provides a gain of 6.3 pounds of useable protein *per capita*; the same amount of subsidy would yield 1.2 pounds of milk protein *per capita*.

Amino acids are produced either by fermentation or by direct synthesis<sup>34-36</sup>. Lower levels of production favour fermentation; at greater concentrations, economies of scale favour synthesis of lysine. Thus, the generator for cost reduction will be the volume of amino acids utilised in animal feeding. Mitsuda and Yasumoto<sup>7</sup> and Durrenmatt<sup>37</sup> showed that the trend of production cost of other synthetic nutrients would suggest a further lowering in cost of lysine. It has been estimated that in large scale production, lysine can be produced for about \$0.50 a pound or less.

Present world lysine production capacity is estimated as 16,000 metric tons annually<sup>15</sup>. It is predicted to reach a level of 60,000-70,000 tons by 1980, primarily because of increased demand as a feed supplement. In some areas demand far outpaces supply; in France the price of L-lysine quadrupled to \$9.30 per kilogram in 1973.

**No substitute for aesthetics** The supplementation of cereals or foods is a completely nutritional manoeuvre. It is not visible; and that is its advantage as it requires no change in eating habits, but by the same token, it is difficult for a government to claim credit for such expenditure without obvious immediately visible beneficial results.

This is not a manoeuvre equivalent to adding meat to the diet, or legumes. Calculations can be made of the meat or milk equivalent in protein added to a nation by fortifying a given cereal grain supply with an amino acid. From a strictly protein point of view, these might be correct. They are not, however, entirely correct because the meat adds calories and other nutrients as well as improving the protein quality of the cereals. More importantly, meat adds variety and a psychological stature to a diet which no amount of fortification can induce. There is no doubt that the use of animal protein as a means of improving cereal protein is the preferred way of improving diets when income is increased.

It is tempting to conclude that where the quest for aesthetic satisfaction in foods predominates, no amount of fortification, be it with amino acids, vitamins, minerals, or protein concentrates, can gain the political support that nutritional considerations warrant. It would, therefore, be expected that fortification will make the greatest initial progress in countries and socioeconomic groups where there has been a modicum of aesthetic satisfaction in foods and where aesthetics are no longer the burning issue. Yet, the appreciation of nutrition as apart from eating as an essential component of public health measures is probably far greater than assumed. It is possible, therefore, that in some areas a purely nutritional measure can gain political support on its own public health merits.

## Projections

The two basic constraints on increased food production are land and energy, and the two are interrelated. Where shortages of the two exist, in the short term the picture is grim. Increases in yield were made possible by engineering food plants to withstand and derive benefit from increased energy inputs. An improvement in the present land constraints requires that plants be engineered for higher photosynthetic yield at constant energy input. The development of new sources of energy which will change the nature of the energy constraints is the most likely long term effect. The practice

of using more nonagricultural inputs to maximise food production will continue. Just as this trend will be reflected in optimum use of water, fertiliser, insecticides and fungicides, so will it by increased usage of nonagricultural food nutrients to enhance the quality of the products of the farm.

It has been estimated that 10,000–20,000 tons of L-lysine will, by 1980, be added to cereals intended for human food<sup>15</sup>. The rapid proliferation of animal analogues from soy and other vegetable protein sources will increase the use of methionine in foods based on the meat and milk model<sup>38</sup>.

Fortification with amino acids alone obviously does not solve all problems of nutrition. It does not provide calories. It does not provide aesthetic satisfaction in foods except where it contributes to the development of meat and milk analogues. It is no substitute for better distribution of wealth either within a country or worldwide. But it is a way of reducing pressure for protein from land. It could be considered by some as a temporary measure to bolster protein supplies until more attractive options become available. But, fortification with amino acids will probably become an essential and regular adjunct of human food systems. In this way, technology will contribute more flexibility to counteract the pressures of greater populations and higher expectations. Nevertheless, the pressure of population constitutes a burden that no amount of technology can expect to overcome indefinitely without a genuine lowering in the quality of life for almost everyone.

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## Nutritional lessons from the Ethiopian drought

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*The drought in Ethiopia and the subsequent international reaction has drawn attention again to the urgent need to consider methods of early assessment of and appropriate responses to such situations. Assessment requires the collection of reliable data; responses should pay attention to established nutritional principles and take account both of the situation as it exists and of the normal dietary patterns.*

THE population of Ethiopia is 25.2 million<sup>1</sup>, of whom at least 90% live by subsistence farming. Ecologically there are a number of different zones, distinguished particularly by altitude and by local variations in climate. In the northern area, where relief efforts were concentrated in 1973 (see Fig. 1), there are broadly three zones: highland (above about 2,500 m), lowland and desert. The farming pattern is mixed: the main food supply is from crops of cereals and legumes in the highlands and lowlands, with increasing amounts of livestock



as the altitude decreases. The two food sources, crops and animals, are interdependent in that oxen are essential for cultivation. The desert people are largely nomadic herders, dependent on cattle and trading with some agriculture, and form a distinct group: they have had limited contact with the mainstream of Ethiopian life, and regard themselves as a separate community.

The land is intensively cultivated and there has been progressive deforestation and soil erosion. Even under normal climatic conditions little excess of grain is produced, particularly in lowland areas, and usually there is a hungry period of one or two months when the previous year's stocks of grain have been exhausted and the next harvest is not yet ready. Excess of livestock creates a little wealth: during times of shortage, animals are sold for meat and hides to buy grain when necessary, albeit at high prices.

The community was particularly vulnerable to climatic change. Between 1966 and 1973 the rainfall was erratic, resulting in poor harvests and inadequate grazing for livestock; local food shortages occurred periodically during this time, leading to population movements and government relief.

There are two annual harvests in most parts of Ethiopia, associated with the main rains (occurring between July and September) and the short rains (usually falling at some time between February and April). The harvest from the main rains, gathered in October to December, provides much of the food supply for the following year, supplemented by crops sown during the short rains; this latter harvest is also of critical importance in providing seed for the main sowing later in the year.

In early 1973 the short rains failed completely in many areas, particularly in Wollo and Tigray provinces. The harvest in late 1972 had been generally poor, and with the subsequent failure of the 1973 early harvest food stocks began to run out, particularly in the lowland areas, from April onwards. In the desert the drought probably had an even more disastrous effect in that little or no grazing remained by the early part of the year. Serious disruptions of the normal market equilibrium occurred. The cost of grain bought by merchants and sold in the large towns rose steeply, while the price obtained for cattle, sold in order to buy grain, fell from Eth\$ 120–150 to about Eth\$ 10. The abattoirs were unable to cope with the influx, so that many cattle, already in very poor condition, died before they could be slaughtered.

In the period from April to August increasing numbers of people, their food supplies in their villages exhausted and their cattle dying from starvation and disease, made their way to the roads and on to the towns. Little information was available as to the conditions in the countryside away from the roads, although it was assumed that many must have died. It was therefore clear at this stage that immediate action was required to prevent widespread malnutrition.

Faced with large numbers of refugees from the drought-affected areas crowded into the towns, sleeping in the open, the local authorities erected temporary shelters. By September, more than 60,000 people were accommodated in 12 shelters in Wollo alone. Food supplies had not yet been established and the construction of latrines was begun through force of circumstance after the shelters were already occupied, resulting in a high incidence of enteric infections. At the beginning of the rains each year there are anyway sporadic epidemics of enteric infections; these and other diseases common in crowded conditions added to the public health problems in the camps.

In April 1973 an Ethiopian government report indicated the seriousness of the drought and food shortage in Wollo, Tigray and northern Shoa provinces. Distribution of grain was begun by the authorities and outside food aid sought; later seed for the next harvest was also distributed. A relief fund and relief committees were established, and considerable

sums of money raised within Ethiopia. It was estimated from official records that nearly 2 million people were affected, and the grain requirement was calculated as of the order of 50,000 tonnes a month. Nothing like this amount was available and the problems of distribution were enormous. Mission and other organisations were receiving reports of drought and starvation, and were also active in providing supplies and help. The drought in the Sahel area was receiving much attention, and in July a meeting in London discussed "Drought in Africa"<sup>2</sup>, including Ethiopia. By September there was widespread concern over the situation in Ethiopia, and further information was forthcoming, notably from UNICEF. At this time national and international agencies became involved, and aid began to arrive in quantity in October. By the beginning of 1974 teams from Britain, Germany, Holland, Switzerland, Australia, the United States and elsewhere were represented, most arriving several months too late to help the Ethiopians with the main problems, which were at their peak before the 1973 harvest. Relief was required both in the camps and in the remote rural areas but until recently was restricted almost entirely to the camps. Here the need was most obvious but their total population after October was not more than 15,000, less than 1% of the affected population.

- defining the situation with respect to the nutritional status of the population, and
- deciding on the appropriate response in terms of food aid.

### Assessing the situation

Most assessments of the situation were obtained from official records in centres of population, and from subjective individual reports. The frequently-quoted figure of 50,000–100,000 deaths (see, for example, ref. 3) was obtained from the records of a few towns, scaled up by an estimated factor to represent the total affected population. Rural conditions could only be assessed from nutritional surveys.

The available information indicates that by October 1973 stock losses of at least 80% had occurred in many areas, that 50–70% of the land was planted, and that the harvest, although better than the previous year, was only moderate. The yield from the harvest was expected to last until around March to May 1974. The reasons for the reduced yield were lack of oxen for ploughing, lack of seed, and poor rainfall.

Results of a survey in Tigray showed that of the rural population the very poor—those without land or livestock—were receiving only about half their required dietary intake, less than 1,000 kilocalories per adult per day and 50 kilocalories per kg per day for children; only one meal a day was usually being eaten. The major part of the population, the farming community, was faring a little better, relatively few of them being of less than 70% weight-for-height, and their dietary intake was marginally adequate, of about 1,500 kilocalories per adult per day, 75 kilocalories per kg per day for children; two meals a day were generally being eaten. The salaried (5–10%) workers were receiving up to 3,000 kilocalories per adult per day.

There was a proliferation of idiosyncratic methods of assessment of nutritional status and much nonquantitative subjective data. Standardisation of survey technique is important and perfectly feasible; nutritional survey methodology is an established aspect of nutritional science<sup>4</sup>.

**Principles of nutritional surveys.** In a situation of developing food shortage time-consuming detailed investigation before action may be unacceptable and crude rapid estimates may be required. Once action is established, aid and more thorough survey work can be concurrent, and indeed survey data is of vital importance in monitoring the effectiveness of the aid.

The primary environmental influences on nutritional status are dietary intake and the occurrence of disease; other

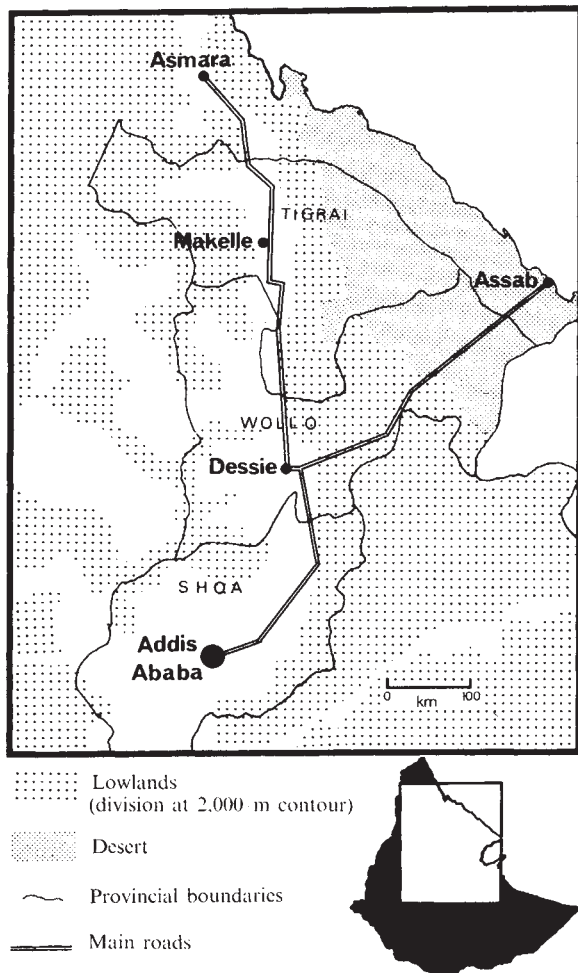


Fig. 1 Northern Ethiopia, showing area known to be affected by the drought in 1973. Highlands are unshaded.

factors—economic, agricultural, social and so on—necessarily exert their effect through either the dietary intake or the disease pattern (see Fig. 2). The nutritional status of the community defines the situation at a point in time but gives no idea as to trends. Current dietary intakes, food stocks and potential supplies provide the primary information as to the likely immediate trend of the nutritional status. The factors affecting nutritional status, shown in Fig. 2, also indicate the points at which action can be taken. Short term efforts, as in relief, are intended to influence dietary intake and disease patterns directly, by feeding programmes and public health measures. Longer term development work attempts to tackle the basic causes of malnutrition.

**Nutritional status.** The two characteristics of protein-energy malnutrition (PEM), wasting and oedema (associated with hypoalbuminaemia) require separate assessment. The wasting component of PEM is readily determined by anthropometric methods. Weight-for-height and height-for-age measurements ideally define both wasting and stunting<sup>5</sup> but for a rapid assessment in an acute situation weight-for-height is the most useful measurement. The level of 70% weight-for-height is frequently taken to be approximately equivalent to the conventional 60% weight-for-age level used to define marasmus<sup>6</sup>. The risk to development of oedema (preclinical kwashiorkor) cannot be assessed by anthropometry; blood samples and determination of serum albumin values are required<sup>7</sup>.

Examination for clinical signs of PEM, together with anthropometry, defines the incidence of severe PEM in the community. The most relevant clinical signs are pitting oedema, generally appearing first on the dorsum of the foot,

and severe wasting, detected on the buttocks as 'baggy pants', when the skin hangs in loose folds; the appearance of this sign occurs at around 70% weight-for-height. Vitamin and mineral deficiencies are assessed by clinical examination.

**Dietary intakes.** Quantitative estimates of dietary intakes can only be obtained by home visits, in which the quantities of food eaten are weighed; nutrient intakes are then calculated from food tables<sup>8,9</sup>. The two important parameters to consider are the total energy intake and the protein adequacy of the diet, estimated as effective protein concentration (Net dietary protein calories % or NDpCal%).

**Community data.** Questionnaires for home visits and for interviews with local officials and at health centres give data regarding current food stocks, livestock losses, expected harvest yields, population movements, patterns of disease and malnutrition and mortality rates.

**Sampling.** The validity and applicability of survey results depends on the sampling methods. Even in preliminary surveys, where the application of strict sampling techniques may be limited by the urgency of the situation, some attempt at randomisation is essential. Stratification by area and within the community can be based on existing demographic, geographical, agricultural and other data, and survey design can then concentrate the effort to manageable proportions. It is inadequate to try to gain an impression without selected home visits, since the most visible members of the community will tend to represent the extremes of nutritional status.

**Conclusions from surveys.** Such survey data define the current nutritional status of the community, the current dietary intakes and hence the trend in nutritional status of the current food stocks (livestock and crops), and the projected food supply in the immediate future.

The usefulness of survey data as a basis for planning depends on its quality. Rapid initial assessments not only become dated but are unlikely to be authoritative enough to support detailed planning.

Whether a situation requires special action (relief) depends on a consideration of the 'usual' situation in such areas. An assessment of a 'famine' situation perhaps depends on the extent to which the normal equilibrium has been disturbed, and in particular on the ability of the farming community to survive and to recover. The levels of hardship which are unacceptable and which should lead to relief being given require urgent consideration by aid-giving organisations.

## Planning

Effective planning of relief is clearly essential but in an urgent situation it may involve particular problems of donor-recipient relationships. The decision to request aid may itself be difficult to take, and the recipient will rightly wish to direct the aid in accordance with its own priorities. These priorities may not agree with those of the donor countries, particularly if the donors are insensitive to local requirements. This can lead to poor coordination and a reduction in immediate and longer term gains. Responsibility for internal administration and coordination lies with the recipient. To ensure a maximum effectiveness, adequate planning and consultation are vital and prior consultation concerning Ethiopia's real needs has often been notably absent.

Successful planning requires that the problem be defined and priorities for action established; where possible these should also advance the longer term development aims. Only then can the appropriate responses be decided.

## Nutritional responses

Food aid requested in 1973 was for both grain and 'high protein foods'. Quantities of grain were based on estimated requirements of 0.4 to 0.8 kg per head per day, supplying about 1,500 to 3,000 kilocalories per head per day. At 0.5



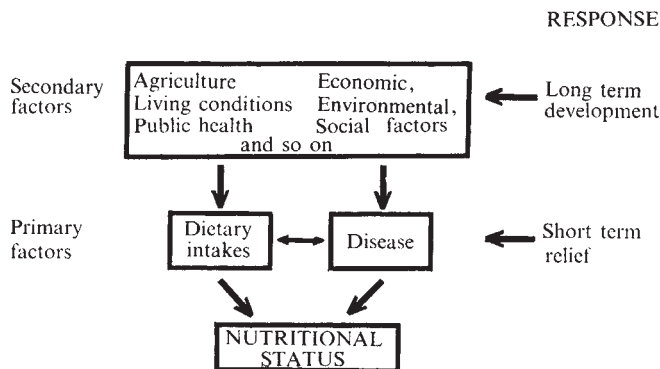


FIG. 2 Exogenous influences on nutritional status and levels at which efforts are directed to improve it.

kg per head per day 1,000 tons would supply 2 million man-days; thus a population of 1 million would require 15,000 tons a month, or 100 5-ton truck-loads a day. The main outside supplies were from international agencies, particularly World Food Programme, which consigned 20,000 tons of grain, of which 10,000 arrived in 1973 (enough to supply the estimated 2 million affected for 10 days, even if it could be distributed.) Further supplies of several thousand tons were redistributed in Ethiopia, and some hundreds of tons of specifically high protein food, designed originally as protein supplements, were also distributed.

It is estimated that about a quarter of the population of the affected areas will require food aid in 1974, and 150,000 tons of grain has been requested accordingly, on the basis of 0.4 kg per head per day; further supplies of protein foods have also been requested from international agencies.

The food supplied to the camps by the government provided 2,000 to 3,000 kilocalories per adult per day, as grain (mainly wheat and maize). At first it was difficult to prepare the food in a form regarded as suitable for main meals but the familiar 'enjera' (made from fermented grain) and 'wot' (a sauce containing legumes, spices and sometimes oil) were later introduced by the Ethiopian Nutrition Institute and were greeted with great enthusiasm.

The choice of foods imported and distributed by most external agencies showed an overemphasis on providing protein, regardless of energy intakes, particularly since the local protein supplement 'Faffa' was available to meet any extra protein requirement. Examples of foods supplied by foreign agencies are sweetened milk, protein biscuits, baby foods packed in individual portions, protein 'tonic' (containing 90% protein of unspecified origin) and, of course, dried skimmed milk. These were neither locally acceptable, nor suitable for treatment or prevention of malnutrition, nor indeed had most of them been requested. Further anomalies, such as the attempted further fortification of the local protein supplement with protein 'tonic', and the provision of exclusively protein supplements (in one case giving twice the protein and half the energy requirements) resulted from the misconception that the malnourished require only protein (and perhaps vitamin pills, which were also used incorrectly).

**Principles of emergency food aid.** In an acute food shortage, food aid is required

- to support the nonmalnourished population—'maintenance feeding', and
- to improve the nutritional status of the malnourished in the population, 'rehabilitation feeding'.

Both quantity and quality of dietary intake need consideration.

Distribution of supplies as widely as possible is clearly preferable to central cooking and feeding, in order to prevent migration and to enable the population to re-establish its normal food supply. Indeed the effective distribution of

supplies can be of critical importance in stimulating recovery from an emergency. The nutritional requirements for both maintenance and supplementary feeding are similar whether the supplies are distributed or prepared centrally, as in camps.

**Maintenance.** Maintenance feeding involves supplying either the total dietary intake for those completely dependent on relief supplies, such as nutritional refugees in camps, or in supplementing an inadequate diet when there are some resources locally available. Nutritional requirements for maintenance are derived from recommended intakes published by FAO/WHO<sup>10</sup>; these are for 3,000 kilocalories per adult per day, and 70–100 kilocalories per kg per day for children, depending on age. As an approximation 2,000 kilocalories per head per day (for example, from 0.6 kg of grain) irrespective of age would be a minimal allowance on a population basis. An effective protein concentration (NDpCal%) of 5% is recommended for all except children of up to 1 year and pregnant and lactating women, who require an NDpCal% of about 6–8. Cereals alone provide an NDpCal% of about 5 and are thus nutritionally adequate to support most of the population. Inclusion of legumes in the diet would increase the protein concentration (see ref. 11) and conform to normal dietary patterns: if 25% of the grain were replaced by legumes the NDpCal% would be at least 8 and thus the protein requirements of vulnerable groups would be met.

Thus the choice of foods for maintenance is based on supplying an energy intake of at least 2,000 kilocalories per head, with an effective protein intake that need be no greater than 8%. (The typical NDpCal% of high protein foods is at least 20%). The choice then depends on the availability, cost, and on the normal dietary patterns and thus acceptability of different foods. For Ethiopia there is no doubt that the main supplies that meet these criteria are cereals, such as wheat, and legumes (peas, beans and so on) with smaller quantities of vegetable oil and spices.

**Rehabilitation.** The nutritional requirements for improvement of nutritional status are higher than for maintenance, particularly to allow malnourished children to catch up lost growth. Increased dietary intakes can be achieved by 'supplementary' feeding of extra food for mild cases of malnutrition or by 'therapeutic' feeding for the most severely malnourished.

Energy intakes in excess of 100 kilocalories per kg per day for children, with a net NDpCal% of 8–10%, are required; in fact, rates of catch-up growth are approximately proportional to the energy intake above about 100 kilocalories per kg per day. When, as in Ethiopia, wasting is the main feature of the malnutrition, the overriding requirement is for increased energy intakes. If the total food intake is reduced extra protein is anyway wasted since it is used for energy. The key to supplying extra energy is to provide vegetable oil.

For supplementary feeding extra rations or meals should be provided for selected groups or individuals. For malnourished children it should be possible to increase energy intakes to 120–150 kilocalories per kg per day, with an NDpCal% of about 8. The choice of foods is similar to that for maintenance feeding, with greater emphasis of acceptability since the malnourished are frequently also sick and may have poor appetites, and the energy concentration of the diet should be increased with oil. In Ethiopia a sauce of high oil content, and thus high energy content, was readily constructed by a modification of the usual recipe. There is no advantage in using premixed foods for supplementary feeding: they have generally low acceptability and are expensive.

Therapeutic feeding requires organisation at an individual level and can only be carried out successfully within some centralised system. It is intended only for treatment of the most severely malnourished, who would normally be hospitalised if possible and who require intensive feeding in order to recover at all. Therapeutic diets are made up from fortified milk mixtures, having an NDpCal% of about 8 and providing

at least 150 kilocalories per kg per day for children. These diets are only applicable when a treatment system can be established, as was done in several camps in Ethiopia (see, for example, ref. 12); in this case use of a premixed diet is justified since the administration of correct and known quantities of nutrients is essential. If a system cannot be established for correct administration there is little value in using therapeutic diets.

### Proper response

The nutritional consequences of the drought in Ethiopia have again (compare ref. 13) demonstrated the need for nutritional surveillance in assessing relief situations, for adequate planning and for appropriate responses in terms of food aid, based on an assessment of the situation, on a consideration of the normal dietary patterns and on sound nutritional principles. A rational and measured approach to relief is possible based on established principles and greater consideration of these could ensure that relief is more effective.

We thank Dr R. G. Whitehead for advice and encouragement.

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## Plate movement and continental magmatism

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*Extensive magmatic and metamorphic events have occurred within the Earth's continental crust far from plate margins. During the last 800 million years Africa has only been host to such events during the breaks in the systematic motion of the African plate. This suggests that sublithospheric heat sources have little effect on a moving plate and can only produce recognisable effects in the upper crust if focused for long periods on the same part of the lithosphere.*

### Magmatic and metamorphic activity in Africa

A wide range of igneous and metamorphic rocks are suitable for radiometric age determination. A plot of frequency of radiometric dates against time (Fig. 1) can therefore be used to indicate fluctuations in the intensity of thermal activity in the upper crust, though this cannot be regarded as an accurate quantitative measure. Nevertheless, Fig. 1 shows clearly that within the past 800

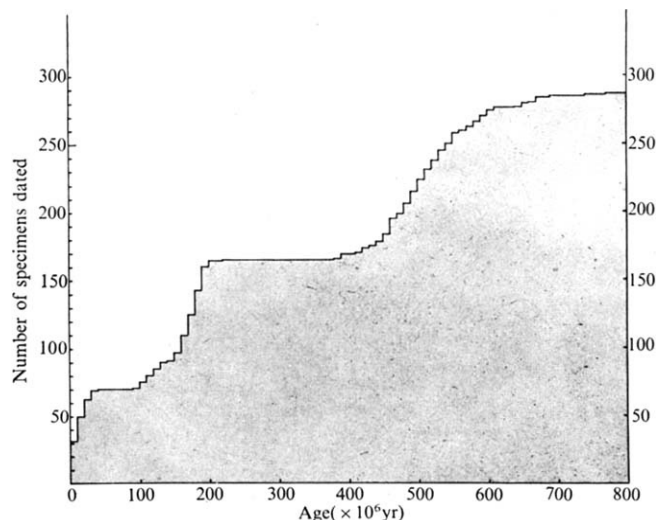


FIG. 1 Cumulative histogram of African radiometric age determinations for the period 0-800 m.y. BP.

SEISMICITY, tectonism and magmatism are now concentrated near plate margins. This may have been so throughout much of geological time. Within-plate continental magmatism and metamorphism are commonly believed to be associated with continental rifting<sup>1-6</sup> but only when rifting proceeds to the stage of transcontinental fracture is the association with plate margins clear.

This paper has three purposes. First, we emphasise that magmatic, tectonic and metamorphic processes have been and are active within continental plates and are by no means exclusively concentrated near plate margins, whether successful or abortive. Second, we note the circumstances in which this activity has occurred, using as an example the magmatic, metamorphic and palaeomagnetic history of a single continental mass, Africa, during the past 800 million years (m.y.). Third, we suggest that this behaviour is to be expected on the basis of thermal convection models of plate motion.





FIG. 2 *a*, Regions (stippled) of Africa which have been affected by the pan-African (650–400 m.y.) tectono-thermal event (after Clifford<sup>7</sup>). *b*, Areas (stippled) of Mesozoic igneous activity (200–100 m.y.) (after Cox<sup>8</sup> and Black and Girod<sup>9</sup>). *c*, Areas of Tertiary to Recent volcanic activity 25 m.y.–present) (after Le Bas<sup>10</sup> and Gass<sup>11</sup>).

m.y. Africa has been the site of three main thermal episodes in the approximate time ranges 650–400, 200–100 and 25–0 m.y. before present (BP). The areas affected by these events are shown in Fig. 2. Magmatic or metamorphic activity between 400 and 200 m.y. BP and between 100 and 25 m.y. BP is sparse<sup>7–11</sup>.

**Pan-African tectono-thermal event<sup>12</sup>.** Igneous and metamorphic rocks over most of Africa (Fig. 2*a*) yield K-Ar ages in the range 650–400 m.y. BP. Metamorphic rocks are the more abundant and their K-Ar ages record a thermal imprint on host rocks which Rb-Sr and U-Pb analyses indicate to be much older<sup>13</sup>. Kennedy<sup>12</sup> recognised that progressive regional metamorphism, ophiolite sequences and molasse-type sedimentation, which commonly characterise orogeny, occur only in restricted areas, such as the Lufilian<sup>14</sup> and Pharusian<sup>15,16</sup> chains in central and western Africa respectively. Elsewhere the event occurs as thermal reactivation of much older basement with extensive metamorphism and production of granitic magmas but little or no deformation. Even, however, if the Lufilian and Pharusian chains were the sites of pan-African subduction the amount of closure was small if palaeomagnetic evidence that Africa maintained the same approximate configuration throughout is accepted<sup>17</sup>.

**Mesozoic magmatism.** Between 200 and 100 m.y. BP magmatic activity was intense and widespread in southern<sup>18,19</sup>, western and possible north-eastern Africa (Fig. 2*b*). For southern Africa, Cox<sup>19</sup> concluded that igneous activity took place throughout the 200–100 m.y. time range and calculated that the erupted lava once covered  $1.4 \times 10^6$  km<sup>2</sup>. These rocks are mainly basaltic, but silicic varieties also occur and these may have been produced by anatexis of sialic crust<sup>19</sup>. Rocks of similar age in West Africa are predominantly silicic with only minor basic volcanic representatives. In both regions the activity was at its greatest between 200 and 180 m.y. BP and from then on generally waned, with occasional resurgences, until it ceased about 100 m.y. BP. Although this activity heralded the rupture of Gondwanaland, magmatism was not restricted to regions close to eventual plate margins. On the contrary, the bulk seems to have occurred within continental regions, which remained unified throughout.

**Late Tertiary to Recent magmatism.** Except in Libya where some volcanic rocks date at 40–50 m.y. BP, and Ethiopia where restricted volcanism might possibly have persisted throughout the Tertiary, rocks of magmatic origin in the age range 100–25 m.y. BP are extremely rare. Volcanic rocks with ages of less than 25 m.y. are, however, abundant (Fig. 2*c*). They are usually found where the underlying and commonly peneplain crystalline basement has been updomed, in places by as much as 3 km (ref. 11). Uplift usually preceded volcanism. Many of these areas of uplift and volcanism, such as Tibesti, the Hoggar and Air massifs, and the Bayuda and Jebel Marra, are completely within the African plate and any fracturing does not extend far beyond the limits of uplift. In eastern Africa the areas of

uplift around the Red Sea and in Ethiopia, Kenya and Tanzania overlap, and the resultant fracture zones join to form the East African Rift System. Beneath these rifts, sialic crust has been markedly attenuated although complete lithospheric rupture has only occurred under the Red Sea and Gulf of Aden. In every case the initiation of uplift and volcanism took place beneath what was then the unified Afro-Arabian lithospheric plate, and constructive plate margins only developed as events progressed.

## Apparent polar wander

The apparent polar wander path for Africa since 750 m.y. BP<sup>17,20,21</sup> is reproduced in Fig. 3. Many of the data were derived from the igneous rocks discussed in the preceding section and are thus well suited to the comparisons discussed here.

The palaeomagnetic poles derived from the Mbozi complex in Tanzania ( $743 \pm 30$  m.y.) and pre-Nama dykes in south-western Africa ( $653 \pm 70$  m.y.) both lie near the present pole. So also do well defined poles from several less well dated rock bodies in the Sudan, Tanzania and Zambia. It therefore seems that around 700 m.y. BP the pole (which is the South Pole) remained near the present North Pole for about 100 m.y., although age uncertainties prevent a positive conclusion.

Poles calculated from many African rocks ranging in age from  $630 \pm 24$  m.y. to late Ordovician (approximately 450 m.y.) lie in or near western Africa. Although the precise form of this part of the polar wander path is not clear, the concentration of poles in this general area is established. It can therefore be inferred that the pole shifted from its position at 700 m.y. during the late Precambrian. The intervening polar path is, however, strewn with a number of pole positions derived from nominally Cambrian rocks from South America and the shift in question might therefore be as late as mid-Cambrian.

The polar path then moves to the vicinity of South Africa. The date of the shift is uncertain and has been variously estimated as late Ordovician<sup>22</sup>, Siluro-Devonian<sup>23</sup>

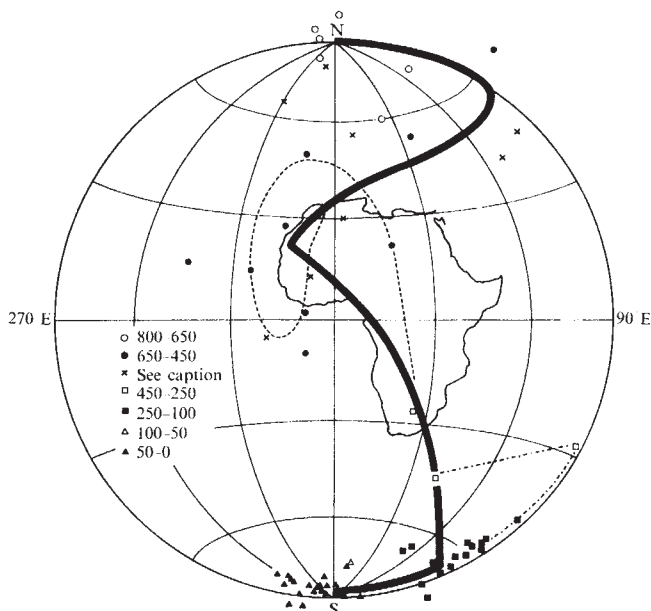


FIG. 3. Palaeomagnetic apparent polar wander path for Africa (thick solid line) from 750 m.y. BP to the present; data from compilations<sup>17,31,39,40</sup> and recent original papers<sup>28,41,42</sup>. Elaborations of parts of this path suggested by Piper *et al.*<sup>17</sup>. (---), and McElhinny *et al.*<sup>21</sup> (— · —) are not well substantiated. South American poles, supposedly of Cambrian age, rotated for closure of the South Atlantic, are denoted by x.

and Devonian<sup>24,25,26</sup>. During the mid-Carboniferous<sup>27</sup>, the pole is known to have moved away from South Africa, and therefore the estimates of the time for which it remained in that area range from only a few million years to rather more than 100 m.y.

Data from all parts of Africa except the north-east, confirm that the pole reached the vicinity of 60°S, 85°E at the beginning of the Mesozoic and remained there, essentially stationary, until about 110 m.y. BP (refs 21 and 24).

It essentially reached its present position by 40 m.y. BP, and it has stayed there ever since<sup>28</sup>. This is reflected in the cluster of poles near the South Pole, shown in Fig. 3. The fact that their mean is some 5° from the geographic pole may be because of the inadequacy of data or persistent non-dipole elements in the palaeomagnetic field, rather than because of any true polar wander or plate motion<sup>29-31</sup>.

There were thus at least three periods of time within the past 750 m.y. during which the palaeomagnetic pole did not move systematically relative to Africa, but instead either stopped or moved circuitously (Fig. 3). These periods occurred around 500 m.y. ago (at least 530–450 m.y. BP and perhaps as long as 630–400 m.y. BP), from 220 to 110 m.y. ago and during the past 40 m.y. Two other possible pauses in apparent polar wander, around 700 and 350 m.y. ago, have also been mentioned but the evidence for them is less secure. It is incorrect to translate apparent motion of the palaeomagnetic pole relative to a crustal plate, into direct estimates of the speed of the plate motion. To do so requires complete knowledge of the tectonic rotation poles, which describe the motion of the plate through time. Nevertheless, it seems realistic to equate episodes of rapid apparent polar wander with episodes of rapid plate motion. Similarly, it can be presumed that a plate has remained essentially stationary during an interval for which all palaeomagnetic poles are statistically indistinguishable.

### Plate motion and thermal activity

The three major African igneous and metamorphic episodes coincide quite precisely with pauses in the systematic motion of the African plate (Fig. 4). In the case of the interval 600 to 400 m.y. the scatter of palaeomagnetic data is such that random or circuitous motion within about 2,000 km of the calculated mean position cannot be ruled out. But for the later two episodes the data suggest that motions of more than a few hundred kilometres are improbable. Each pause in systematic motion follows a period of substantial shift, and is succeeded by a new shift in a different direction. Despite the statistical uncertainties in radiometric dating and in palaeomagnetic estimation of latitude, and the impossibility of determining ancient longitude from palaeomagnetism, this remarkable time correlation is unlikely to have arisen by chance.

The evidence thus suggests that changes from one regime of plate motion to another were not accomplished rapidly. During the transitions, motions were either more complex or ceased altogether. This conclusion is intuitively sensible considering the enormous mass inertia of the system and has been advanced<sup>43</sup> to explain a correlation between bends in the Canadian polar wander path and orogenic events.

It also seems that extensive magmatism and metamorphism within the African plate only developed during these transitional periods. This might be anticipated when the enormous thermal capacity of the system is considered. From the general character of the global geological record it is clear that the lower crust and uppermost mantle are normally below their solidus temperatures. As the heat produced within the continental and oceanic lithosphere accounts for only about 65% and 3% respectively of the observed surface heat flow<sup>32</sup>, the fundamental requirement for magmatism

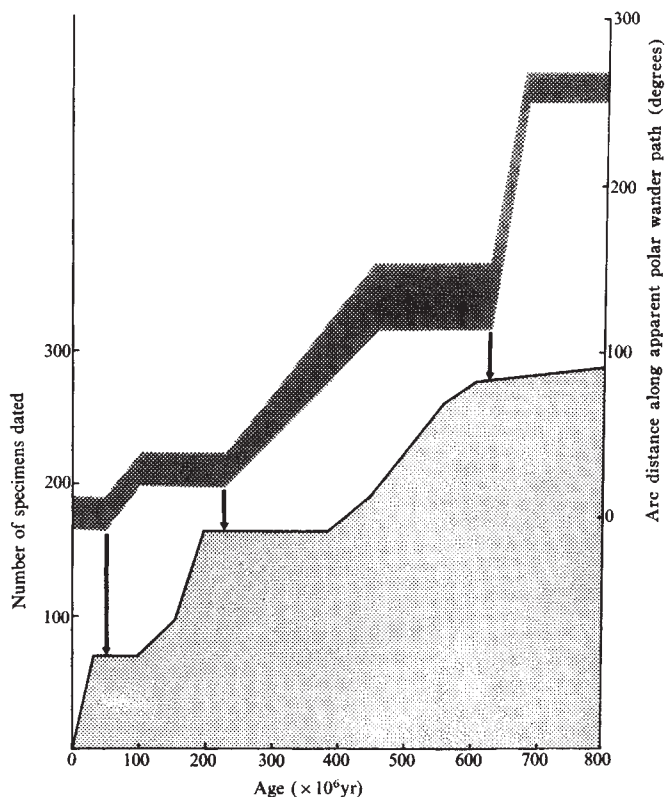


FIG. 4 Above, schematic representation of the erratic character of apparent polar wander (generalised from Fig. 3). Below, simplified cumulative frequency histogram of radiometric age determinations (based on Fig. 1). Vertical lines emphasise the correlation between the breaks in apparent polar wander and the start of thermal activity.

and metamorphism at high levels in the crust is a substantial heat source beneath the lithosphere. The fact that these effects are regionally restricted at particular times indicates that such sources are unevenly distributed. Our observations also imply that the heat sources have maximum effect when they are applied beneath the same region of lithosphere for many millions of years. Only then does large scale mantle or crustal anatexis occur. These in turn give rise to thermal metamorphism within a few kilometres of the surface. Whether the heat sources begin as localised hot spots of asthenospheric or deeper origin<sup>3,4,32-35</sup>, or as more extensive asthenospheric upcurrents of a nascent plate-driving system, is not critical in this context. It is, however, pertinent to note that the ultimate source of the Mesozoic Karroo magmatism might have been as deep as 500 km (ref. 19).

For the thermal source and the target to be fixed relative to each other, either both must move together<sup>36</sup> or, as is contended here both must remain stationary with respect to the rotation axis of the Earth.

The identification of linear age trends along particular lines of igneous activity<sup>37</sup> does not invalidate our main conclusions. Such trends are rare, have been disputed<sup>36,38</sup> and in any case are short when compared with the dimensions of the igneous provinces as a whole.

If thermal conduction were the only heat transfer mechanism, there would be a time lag of the order of  $10^8$  yr before sublithospheric thermal events permeated to the surface. The effects could be localised and accelerated by convective heat transfer in the asthenosphere and by upward magma migration. Although it is tempting to suggest that time lags are in fact observed between apparent cessation of African plate motion at 220 and 40 m.y. and the surges of subsequent igneous activity at 200 and 25 m.y. respectively (Fig. 4), this inference is probably unjustifiable on the



evidence available. Certainly no such effect is evident for the pan-African event.

Nevertheless, our analysis demonstrates that widespread continental igneous activity could be the result of stationing a plate over a heat source of a kind which could, potentially, cause continental breakup. It is suggested that thick continental lithosphere will not inevitably break in response to such heating. Instead it may become locally elevated, the thermal gradient may become enhanced and extensive anatexis may be produced as well as widespread thermal metamorphism of pre-existing rocks of the upper crust.

Received November 29, 1973; revised January 31, 1974.

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## Further evidence of Lower Pleistocene hominids from East Rudolf, North Kenya, 1973

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*Twenty new hominid specimens were recovered from the East Rudolf area in 1973. New evidence suggests the presence of at least three hominid lineages in the Plio-Pleistocene of East Africa.*

THIS is a report of the 1973 field season at East Rudolf, Kenya, where the East Rudolf Research Project (formerly Expedition) has now concluded its sixth year of operations. Eighty-seven specimens of fossil hominid were collected<sup>1</sup> from the area during 1968–72; a further twenty specimens were recovered between June and September 1973 from the Upper, Lower and Ileret Members of the Koobi Fora Formation<sup>2</sup>. Exploration to the south of Koobi Fora was begun in 1972 and continued in 1973. No hominids have yet been found

in the limited exposures of the Kubi Algi Formation. A notice of two specimens—KNM-ER 1510 and 1590—that were previously<sup>1</sup> mentioned only by number, is included in this report. The 1973 hominids are not here attributed to genera as there are still no clear generic diagnoses available for fossil hominids. With a few exceptions, previous attributions for the East Rudolf hominid collection remain satisfactory.

Archaeological investigation during 1973 was extended under the direction of G. Ll. Isaac, with J. C. W. Harris who conducted major excavations at several sites in the Upper Member of areas 130 and 131. Limited excavation, but extensive prospecting, in the Lower Member produced sufficient results to support further searching for artefacts below the KBS Tuff.

TABLE 1 1973 hominid collection from East Rudolf

| KNM-ER No. | Specimen                                                                               | Area | Member |
|------------|----------------------------------------------------------------------------------------|------|--------|
| 1800       | Cranial fragments                                                                      | 130  | Lower  |
| 1801       | Left mandible, P <sub>4</sub> , M <sub>1</sub> , M <sub>3</sub>                        | 131  | Lower  |
| 1802       | Left mandible, P <sub>1</sub> -M <sub>2</sub> and right P <sub>3</sub> -M <sub>2</sub> | 131  | Lower  |
| 1803       | Right mandible fragment                                                                | 131  | Lower  |
| 1804       | Right maxilla, P <sup>3</sup> -M <sup>2</sup>                                          | 104  | Upper  |
| 1805       | Cranium and mandible                                                                   | 130  | Upper  |
| 1806       | Mandible                                                                               | 130  | Upper  |
| 1807       | Right femur shaft                                                                      | 103  | Upper  |
| 1808       | Associated skeletal and cranial fragments                                              | 103  | Upper  |
| 1809       | Right femur shaft                                                                      | 127  | Lower  |
| 1810       | Proximal left tibia                                                                    | 123  | ?Lower |
| 1811       | Left mandible fragment                                                                 | 123  | ?Lower |
| 1812       | Right mandible fragment and left I <sub>1</sub> and M <sub>1</sub>                     | 123  | Lower  |
| 1813       | Cranium                                                                                | 123  | ?Lower |
| 1814       | Maxillary fragments                                                                    | 127  | Upper  |
| 1815       | Right talus                                                                            | 1    | Upper  |
| 1816       | Immature fragmented mandible                                                           | 6A   | Upper  |
| 1817       | Right mandible                                                                         | 1    | Upper  |
| 1818       | I <sup>1</sup>                                                                         | 6A   | Upper  |
| 1819       | M <sub>3</sub>                                                                         | 3    | Upper  |
| 1820       | Left mandible with M <sub>1</sub>                                                      | 103  | Upper  |

During the palaeontological survey, which was supervised by J. M. Harris, all identifiable fragments from certain horizons were collected; new species were recorded and some primate remains were recovered during a limited survey of the Kubi Algi Formation. A detailed account of the East Rudolf fauna will be presented upon conclusion of current studies, but there are clear indications that at times the palaeoenvironment differed from that of the lower part of the Shungura Formation of the Omo Valley in Ethiopia.

In the geological studies, emphasis was placed on microstratigraphy and palaeoenvironmental reconstruction. B. Bowen supervised a study of the Lower and Upper Members of areas 130 and 131 which included confirming the stratigraphic relationships of the cranium KNM-ER 1470. The complete section of the Koobi Fora Formation exposed in area 102 was studied by a group from Dartmouth College, New Hampshire, under G. Johnson. A. K. Behrensmeyer completed a preliminary geological investigation of the hominid sites, noting depositional environments and possible association of fauna; further studies are planned.

I. Findlater extended mapping of tuffaceous horizons to the south of Koobi Fora and collected samples for isotope dating. A series of dates has been obtained from material collected during 1972 (unpublished work at Miller, Findlater, Fitch and Watkins). Palaeomagnetic studies complement those of 1972 and there are sufficient data for internal correlations to be made<sup>2</sup>.

## Hominid collection

Specimen KNM-ER 1590, reported previously<sup>1</sup>, consists of dental and cranial fragments which were collected from area 12, some metres below the KBS Tuff. Both parietals, fragments of frontal and other pieces of cranial vault, the left deciduous *c* and *dm*<sup>2</sup>, the left and right unerupted C, P<sup>3</sup> and P<sup>4</sup>, and the erupted left and right M<sup>1</sup> and left M<sup>2</sup> were recovered. Although the cranium is immature, it was large with a cranial capacity as great as that determined for KNM-ER 1470. The parietals may show some deformation but, in any event, they suggest that the cranium was wide with a sagittal keel.

KNM-ER 1510, also reported previously<sup>1</sup>, includes cranial and mandibular fragments. The specimen is poorly mineralised and further geological investigation at the site indicates a Holocene rather than an early Pleistocene provenance as originally thought.

The 1973 hominids and their stratigraphical positions are listed in Table 1. Specimens from area 123 are rare, and their stratigraphical position relative to the Upper and Lower Members of the Koobi Fora Formation needs clarification.

A well preserved mandible (Fig. 1), KNM-ER 1802, was discovered by J. Harris *in situ* below the KBS Tuff in area 131. The dentition is only slightly worn, and fragments of both M<sub>3</sub> crowns suggest that death occurred before full eruption. The canines and incisors are represented by roots and by alveoli filled with matrix. The mandible shows some interesting features—moulding of the mandibular body, absence of a strong post-incisive planum, the development of a slight inferior mandibular torus and the distinct eversion of the mandibular body when viewed from below.

A weathered mandible, KNM-ER 1801, bears some resemblances to KNM-ER 1802, but its worn dentition and loss of surface bone prevent direct comparisons. The relative proportions of the molars and premolars may have been exaggerated by interstitial wear.

A crushed maxillary fragment, KNM-ER 1804, with P<sup>3</sup>-M<sup>2</sup> preserved was discovered by R. Holloway. The teeth are complete but worn.

A skull (cranium with associated mandible), KNM-ER 1805, was discovered by P. Abell *in situ* in the BBS Tuff complex in area 130. The specimen is heavily encrusted with a hard matrix and will require careful preparation before its morphology is revealed. Comments here are thus preliminary. The cranium is in pieces which fit together. After preparation, it should be possible to determine the endocranial capacity; at present, a volume of 600–700 cm<sup>3</sup> is suggested. The supraorbital region, much of the face and the greater part of the basi-cranium have not been preserved. The postorbital region is preserved and the minimum breadth is approximately 90 mm. No distinct temporal lines cross the frontal area although they can be discerned and are still apart at the bregma. There are distinct parasagittal crests. The nuchal attachments are very distinctive and protrude to form a wide bony shelf. The palate is intact; all the teeth are preserved except for the right P<sup>4</sup> and the left I<sup>1</sup>. The mandible, small and distinctly robust, is represented by both sides of the body but, except for the right M<sub>2</sub> and M<sub>3</sub>, the tooth crowns are missing. The ascending rami are not preserved. The right M<sub>3</sub> and M<sub>2</sub> are well worn but small. The upper dentition shows wear on all the teeth, including M<sup>3</sup>.

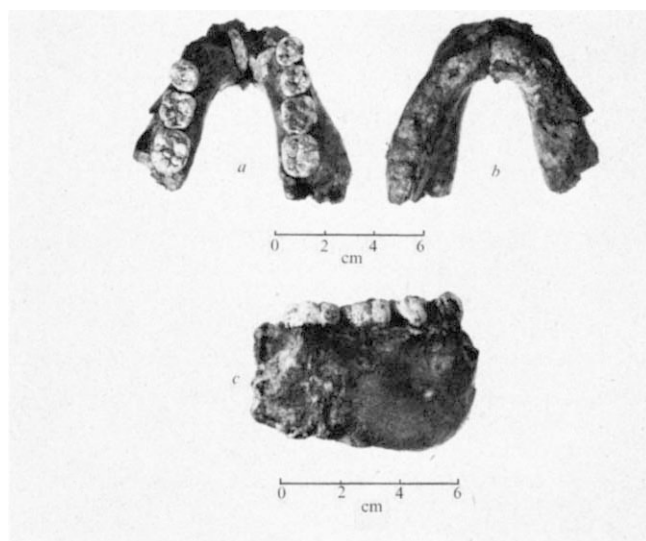


FIG. 1 Mandible, KNM-ER 1802. a, Superior view; b, inferior view; c, right lateral view.



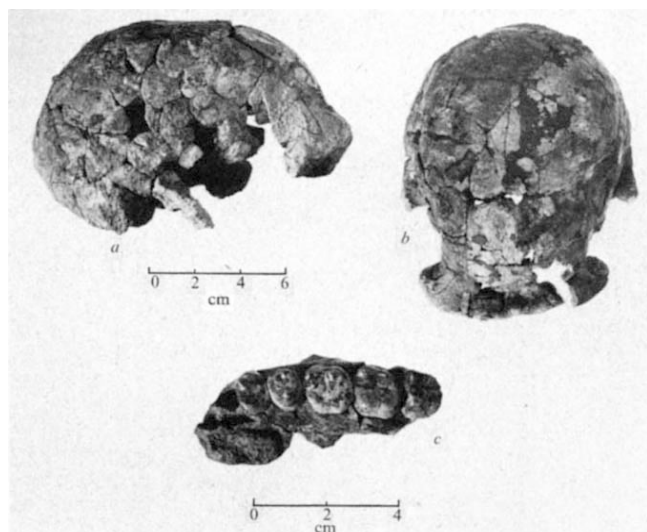


FIG. 2 Cranium, KNM-ER 1813. *a*, Right lateral view; *b*, superior view; *c*, occlusal view of left side of palate.

A large mandible, KNM-ER 1806, was discovered by Meave Leakey at the same site and horizon as was KNM-ER 1805. There are no tooth crowns preserved and the ascending rami are missing in this otherwise complete specimen. The mandible is typical of the large East Rudolf hominid that I have previously attributed to *Australopithecus*.

A fragmented specimen, KNM-ER 1808, was discovered in area 103 by Kamoya Kimeu. The specimen includes maxillary and mandibular teeth, cranial and mandibular fragments, a fragment of atlas vertebra, the distal half of a femur lacking the condyles, a large segment of humerus and other postcranial fragments. There is little doubt that the various pieces are from one individual and further sieving and excavation will be undertaken in the hope of recovering more material.

A cranium, KNM-ER 1813 (Fig. 2), was discovered *in situ* by Kamoya Kimeu in area 123. The specimen was fragmented but has been partially reconstructed. Plastic deformation is evident. The cranium is partly covered with a thin coat of matrix and considerable preparation is needed before a detailed description can be attempted. The endocranial volume is likely to be small; a figure of approximately 500 cm<sup>3</sup> is suggested on the basis of comparative external measurements. Other interesting features include the curvature of the frontals, a postglabella sulcus and the small dentition. The maxilla has well preserved teeth, P<sup>3</sup>-M<sup>3</sup>, on the left side, but on the right side only the tooth roots and the complete crown of M<sup>3</sup> remain. Both canines and lateral incisors are present but the central incisors seem to have been lost before fossilisation. Both sides of the maxilla fit together to give the form of the dental arcade. The right maxillary fragment includes the malar region and connects with the lateral margin of the right orbit.

Other specimens recovered during 1973 are listed in Table 1 and will be described in detail after studies are completed.

### Significance of the 1973 collection

In previous reports<sup>1,4-7</sup>, the East Rudolf hominids were assigned to *Australopithecus*, *Homo* or indeterminate (the last category included both very fragmentary specimens and those of uncertain taxonomic rank).

The East Rudolf specimens that have been attributed to *Australopithecus* span a period of time from 3 million years to just over 1 million years with apparently little morphological change. This form is likely to be the same species

as *A. boisei*<sup>8</sup>; it also shows similarities with *A. robustus* from southern Africa. A Pliocene origin is suggested for this specialised group.

Specimens attributed to *Homo* have been recovered from deposits covering a similar time span, but these show greater morphological variability. Those recovered from the Ileret Member seem to differ from those recovered from the Lower Member of the Koobi Fora Formation. The suggestion that a large brained, fully bipedal hominid was living at East Rudolf 3 million years ago was put forward after the 1972 discoveries<sup>7</sup>. This point of view is supported by the cranial fragments, KNM-ER 1590, also from below the KBS Tuff, and this specimen is provisionally attributed to *Homo*.

The 1973 collection from East Rudolf raises many questions. The new mandible, KNM-ER 1802, could be considered as belonging to the same genus and species as KNM-ER 1470 and 1590. There are striking similarities between the dental characters of KNM-ER 1802 and some specimens from Olduvai Gorge such as the type mandible of *Homo habilis*, OH 7. Although the suggested cranial capacity for *H. habilis* is appreciably smaller than that determined for KNM-ER 1470, the discrepancy may be due to the fragmentary material upon which the former estimates were made. I consider that the evidence for a 'small brained' form of *Homo* during the Lower Pleistocene is tenuous.

The cranium, KNM-ER 1813, may prove to be quite distinct from the robust australopithecines and from *Homo*, as represented by KNM-ER 1470. The dentition is 'hominine', yet the cranial capacity appears small. The cranium has some of the features seen in the gracile, small brained, hominid *Australopithecus africanus* Dart, from Sterkfontein.

I have previously questioned the validity of a distinct gracile species of *Australopithecus*<sup>6</sup>, but this new evidence reopens the possibility of its existence. Some authors<sup>9,10</sup> have suggested that *Homo habilis*, particularly OH 24, shows features typical of *Australopithecus africanus*. My suggestion here, that *H. habilis* may have affinities with KNM-ER 1470 and 1590, refers only to OH 7 and OH 16. Features of the calvarium of OH 24 show similarities with KNM-ER 1813. The size and morphology of the teeth of the two specimens are alike and the cranial capacities may also be comparable<sup>11,12</sup>.

The skull KNM-ER 1805 is undoubtedly important, but its interpretation is enigmatic. Its relatively large cranium bears sagittal and nuchal crests but has small teeth; this combination is in contrast to all the specimens previously recovered from East Rudolf.

In any consideration of the affinities of the East Rudolf hominids, the question of sexual dimorphism must not be overlooked. There does seem to be evidence for quite marked sexual dimorphism in one hominid group as demonstrated by the East Rudolf crania, KNM-ER 406 and 732<sup>5</sup>. Unfortunately both crania lack teeth so that the dental characteristics of the alleged female are far from clear.

The possibility of more than two contemporary hominid lineages in the Plio-Pleistocene of East Africa may now have to be recognised, whereas previously one, or at most, two forms were assumed. The attribution of isolated teeth may thus become even more difficult than it is now. Postcranial identifications likewise may be difficult, although the proximal femoral material continues to suggest a morphological dichotomy.

I suggest the following as a basis of nomenclature for Plio-Pleistocene hominids. One genus would include much of the material currently referred to *Australopithecus robustus* and *A. boisei*. A second genus would incorporate many of the gracile specimens from Sterkfontein presently referred to *A. africanus*, perhaps certain specimens from East Rudolf including KNM-ER 1813, and possibly some from Olduvai such as OH 24. A third genus, *Homo*, would incorporate specimens such as KNM-ER 1470 and 1590 from East Rudolf

and possibly OH 7 and OH 16 from Olduvai. Some material from South Africa might also be considered within this last category together with later specimens from Olduvai and East Rudolf. The unusual mandible KNM-ER 1482<sup>1</sup>, together with the specimen from the Omo area referred to *Paraustralopithecus*<sup>13</sup> and certain other specimens from Omo which are contemporary with the three groups just mentioned, could be considered a fourth form—a remnant of an earlier population that disappeared during the early Pleistocene. All these forms may be traced back well beyond the Plio-Pleistocene boundary.

These remarks are necessarily speculative. A more detailed review of hominid systematics is being prepared in collaboration with B. A. Wood. The wealth of data now available presents a new era in the study of early man. The complexities of dealing with the enlarged sample are challenging, and isolated studies on specific specimens must be replaced by exhaustive studies on all the fossil hominid evidence.

I should like to express appreciation for the financial backing provided by the National Geographic Society, the National Science Foundation, the W. H. Donner Foundation and others. The support and encouragement of the National Museums of Kenya and the Kenya Government

made the research possible. Members of the East Rudolf Research Project are too numerous to thank individually but all play a part in a successful field season and are thanked along with those who made important discoveries. I would also express thanks to my wife Meave who, as always, provided invaluable assistance both at the museum and in the field.

Received January 9, 1974.

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## LETTERS TO NATURE

### PHYSICAL SCIENCES

#### Years of peak astronomical tides

THIS investigation stems from an obscure statement by the late D. H. Macmillan in a semi-popular book on tides<sup>1</sup>. He stated (page 48) that the years when the tide-raising forces reach their greatest peak values occur at intervals of very roughly 16 centuries, and gave a sequence of dates starting with 3500 BC and ending with 1433 and 3300 AD. He did not describe the exact terms of the calculation but merely suggested that they involved coincidence of perihelion (closest approach of the Earth to the Sun) and zero solar declination (Sun crossing equator), which is in fact impossible within the given range of dates. (The longitude of perihelion crosses the equator at intervals of about 105 centuries and will next do so in 6581 AD). Further, if one computes the lunar and solar elements for 3300 AD, for example, one finds that they are not particularly favorable for large tides.

To clarify the situation I must first resolve a paradox. By simple consideration, the greatest tide-raising forces must occur when the Moon and the Sun are in conjunction with the Earth and are at their closest respective distances. But it is also common knowledge that the greatest 'perigee-spring tides' (spring tides coinciding with a close approach of the Moon) occur at the equinoxes when the Sun crosses the equator), which as mentioned above cannot coincide with the closest approach to the Sun around the present millennium. The solution lies in the fact that the equinoctial condition maximises only the semi-diurnal components of the tide at the expense of the diurnal components. Since most parts of the ocean have a magnified response to the semi-diurnal frequencies, one must therefore look to the equinoxes

rather than perihelion for the greatest ocean tides. Then, all the major 'harmonic constituents'  $\mu_2$ ,  $2N_2$ ,  $N_2$ ,  $M_2$ ,  $L_2$ ,  $S_2$ ,  $K_2$  are nearly in phase, and the only loss due to the slightly more than minimum distance of the Sun would be an unfavourable phase for the constituent  $T_2$ , which is practically negligible because it has a typical amplitude of 2 or 3 cm of water (about 6% of the mean solar tide). On the other hand, if we are more interested in the maxima of the tide-raising forces themselves, or in movements of the Earth's crust which respond to them more directly than the ocean, then position relative to the equator becomes irrelevant and we should confine attention to the times of perihelion. The dates when both types of maxima occurred are considered separately below.

I restricted the computations to the years 1 to 4000 AD. Outside this range, the accepted formulae become unreliable and interest in the problem diminishes. For the times of peak tide-raising forces, the computer was asked to select the years for which the following conditions all occurred simultaneously: (a) Earth at perihelion, (closest to Sun); (b) longitude of Moon's perigee within a small angle  $\epsilon$  of perihelion or of its converse (aphelion); and (c) longitude of Moon's ascending node within  $\epsilon$  of perihelion or of its converse. The Moon's actual (mean) longitude was also computed at the given times, to check whether the angular limits were not violated by the time needed to adjust to the nearest appropriate conjunction with the Sun (the conjunction very near to perigee). Standard formulae, as found in the *Astronomical Ephemeris*, were used for the four relevant mean longitudes. With  $\epsilon = 5^\circ$ , perihelion satisfied the above conditions in only the following years: 1340, 1433, 1526, 1619, 3182, 3275, 3368 and 3461.

The appearance of the year 1433 shows that this was the type of calculation behind Macmillan's dates, but 3300 does not appear and there is no suggestion of a 1,600 yr cycle. On



the contrary, the salient features are sequences of exactly 93 yr occurring in groups of four in alternate millennia (also confirmed by tentative calculations for the first millennium BC). The 93 yr period arises because it is close to 5 times the 18.61 yr period of revolution of the Moon's nodes and 10.5 times the 8.85 yr period of perigee—condition (b) permits half periods. But, as the basic periods are strictly incommensurate, one cannot expect any exact cycle of repetition.

The configurations on most of the eight occasions above are, however, somewhat spoilt by the longitude of the Moon itself. If the Moon's 'anomaly' (its angular distance from perigee) is more than about  $60^\circ$  at the given instant, an adjustment of 5 to 15 d is required to define the optimum position, during which time the Sun moves by as many degrees as days. The only cases for which the Moon's anomaly and its angular distance from the Sun are less than  $60^\circ$  are in the years 1340 and 3182. Only in these two years does the peak tide-raising force truly occur within  $5^\circ$  of perihelion, perigee and a node.

Turning now to the case of peak semi-diurnal tides, the relevant computations follow a similar pattern, except that in the descriptions of conditions (a), (b) and (c), 'perihelion' is replaced by 'equinox'. (That is, I take the Sun over the equator instead of at its least distance from Earth.) Since equinoxes occur twice as often as perihelion, the conditions tend to be satisfied more frequently. The interval of 4.4 yr between passages of the longitude of perigee past the equinoxes, (condition (b) has been observed in the study of extreme low (or high) predicted sea levels<sup>2</sup>. It might be thought that condition (c) should be confined to the autumnal equinox, so that the 18.61 yr 'nodal modulation' gives the constituent  $M_2$  its greatest amplitude, but in fact when the Moon passes the equator,  $M_2$  is in phase with  $K_2$ , which has a correspondingly reduced amplitude, and other 'nodal modulations' are similarly self-cancelling. So both equinoxes are equally valid in condition c.

With  $\epsilon = 5^\circ$ , the three conditions are satisfied on 27 occasions in 1 to 4000 AD. Only those for which the Moon is within  $60^\circ$  of the appropriate conjunction are shown in Table 1, for reasons stated earlier. V represents the vernal and A the autumnal equinox.

TABLE 1 Most likely dates of peak tides

|      |                                  |                         |       |
|------|----------------------------------|-------------------------|-------|
| 135A | 1020V<br>1113V<br>1745A<br>1922A | 2192A<br>2732V<br>2825V | 3002V |
|------|----------------------------------|-------------------------|-------|

This time no cyclic pattern emerges, either in Table 1 or in the complete list of 27 dates. Intervals of 93 yr occasionally appear (for example, 1020–1113 and 2732–2825) for the same reason as before, but the use of the fixed reference of the equinox instead of the slowly moving perihelion renders the 93 yr cycle less persistent.

The conditions for 1922 were particularly favourable, since the Moon was less than 1 d off 'change' (the instant when 'New Moon' commences) at the time of the equinox and the other relevant angles were also very small. Accurate computations of the lunar and solar ephemerides<sup>3</sup> show that the peak semi-diurnal tide-raising force occurred 1922 September 21 0539 h GMT, with amplitude 1.8728 times the mean lunar amplitude ( $M_2$ ) or 1.2782 times the mean 'spring' amplitude ( $M_2 + S_2$ ). Actual ocean tides usually reach their peak a little later than the peak tidal forces, because of the peculiarities of their dynamic response. Among tide-gauge records from 1922 which are ready to hand, Brest reached a peak of  $1.72 \times M_2$  range, ( $1.28 \times$  mean 'spring' range) on the tide of September 22 p.m. to

September 23 a.m., while Newlyn reached  $1.64 \times M_2$  range ( $1.22 \times$  mean 'spring' range) on the same tide.

Dynamic response may well, of course, cause the tide at a given place to reach its ultimate peak in other years. Places with large diurnal components would again require a quite different set of conditions.

This subject has recently taken on a surprising topicality because of widespread press reports (some grossly exaggerated) of forecasts of unusually large tides for certain days in 1974. The situation is that on January 8 the Moon's perigee occurred within less than 2 h of Full Moon, while the Earth was within 5 d of perihelion. This is close to the conditions for peak tide-raising forces but the fact that the Moon's line of nodes was some  $16^\circ$  away from the Sun disqualifies it from my more rigorous conditions. Being some 72 d off the spring equinox, the (semi-diurnal) oceanic tides are not extraordinarily large and a glance through the tide tables of recent years confirms that equally high and low waters were forecast, for example, for 1966 and 1972. The Full Moons of February and March 1974 occur successively later than perigee, but the approach of the equinox of March 21 compensates to make the spring tides about equally high in all three months. A similar sequence occurs in July, August and September when perigee occurs near New Moon but this time the Sun is not unusually close. The year 1974 is perhaps remarkable for its number of fairly large tides but if one were to compute the occurrences of equal or greater tides in 4,000 yr, one would obtain a very long list.

I thank Mr C. R. J. Davey for calling my attention to the inconsistencies in Macmillan's account of the subject<sup>1</sup>.

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Received October 2 1973; revised January 23, 1974.

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## Creation of an artificial lunar atmosphere

THE tenuous nature of the lunar atmosphere is maintained by rapid loss of gases released at the lunar surface. But would such rapid loss still occur if the density of the lunar atmosphere were greatly increased from its present value? Here I seek to answer this question by evaluating quantitatively the atmospheric loss mechanisms operating in the lunar environment. One conclusion is that if the density of the lunar atmosphere is increased, a point can be reached where loss occurs so slowly that it is negligible over human time scales (that is, exponential decay lifetimes are greater than hundreds of years). So the present lunar 'vacuum' is a fragile state that should be treated carefully if it is to be preserved or could be modified if so desired.

The present lunar atmosphere has measured surface number densities of less than  $10^7 \text{ cm}^{-3}$  (refs 1–3) and a total mass of approximately  $10^4 \text{ kg}$ . As a result the entire lunar atmosphere is a collisionless exosphere in which the constituents travel along ballistic trajectories between collisions with the surface. When they are re-emitted from the surface, a fraction have sufficient thermal energy to escape from lunar gravity. The exponential lifetime against such thermal escape is about 3,000 yr for atoms of mass 20 (ref. 4). A more efficient loss mechanism, however, is operating in the lunar environment<sup>2,4,5</sup>. This involves the interplanetary electric

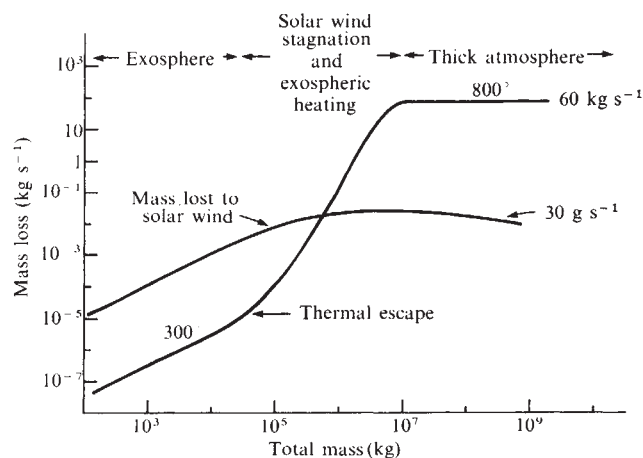


FIG. 1 Loss rates for an oxygen (mass 16 a.m.u.) atmosphere. Molecular oxygen has a smaller thermal escape rate but dissociation would occur in the upper atmosphere. The thick atmosphere thermal loss has probably been overestimated since I have assumed the entire planetary exosphere to be isothermal. Also, exospheric heating may not occur as rapidly as indicated. At a total mass of  $10^8$  kg the lunar atmosphere approaches a constant rate of mass loss. Temperatures are in K.

field resulting from the motion of the solar wind. An atmospheric ion formed through photoionisation by the solar ultraviolet or by collisional ionisation by the solar wind is accelerated initially in the direction of the interplanetary electric field. Half of the atmospheric ions are thus lost into space and half are driven into the surface. The time constant for exponential decay of the atmosphere by this process is determined by the ionisation lifetime (typically  $10^6$  to  $10^7$  s). Thus, gases added to the lunar exosphere are lost within weeks to months. This rapid loss has been confirmed by observations of Lunar Module exhaust gases by the Apollo Suprathermal Ion Detector Experiment<sup>6,7</sup>. There is, however, a limit to the amount of atmosphere that can be swept away by the solar wind. If the atmosphere is dense, newly formed ions of atmospheric origin load down the solar wind flow and divert it around the planet. Thermal escape may then become the dominant loss mechanism.

A quantitative evaluation of these atmospheric loss mechanisms is shown in Fig. 1 for an oxygen atmosphere. The exospheric loss rate to the solar wind was calculated assuming that the total ionisation rate was  $5 \times 10^{-6}$  ion per atom  $s^{-1}$  and that half of the exospheric mass was on the dayside. The limit to mass lost to the solar wind for a thick atmosphere is taken as equal to the solar wind mass flux through the lunar cross section ( $\sim 30$  g  $s^{-1}$ ), since critical mass loading of the solar wind occurs if ions are added at a rate comparable to the solar wind flow<sup>8</sup>. Venus and Mars each lose about 10 g  $s^{-1}$  to the solar wind<sup>8,9</sup>, which is 1% (Venus) and 20% (Mars) of the mass flux of the solar wind through their cross-sectional area. The thermal escape rate<sup>4</sup> was calculated with 300 K as a weighted average of the lunar surface temperature. Absorption of the solar wind and ultraviolet by a thick atmosphere results in exospheric heating and more effective thermal evaporation. As the atmospheric density is increased, however, the exospheric base rises above the surface and the mass lost to thermal evaporation becomes constant. Consequently, the lifetime increases linearly as the atmospheric mass increases. It is not yet certain to what density the lunar atmosphere can be increased; this problem is being investigated.

Figure 2 shows the ultimate atmospheric masses which result from various constant gas addition rates,  $Q$ . The effect of inducing a transition from an exosphere with rapid loss to a thick atmosphere with slow loss is illustrated by considering

the result when the gas source is shut off ( $Q = 0$ ). The thick atmosphere decays with an exponential lifetime of several hundred years whereas the thin exosphere decays in a few weeks. It is estimated that a constant addition rate of approximately 100 kg  $s^{-1}$  is required to transform the lunar exosphere into the long lived atmosphere state. Although the total atmospheric mass would increase to  $10^8$  kg, the surface density would still be low compared to densities in the terrestrial atmosphere.

These considerations do not include possible loss of gases to the lunar surface. Adsorption of gases such as nitrogen oxygen and carbon monoxide by the lunar soil is reversible and permanent retention (chemisorption) generally does not occur<sup>10-13</sup>. The adsorptive capacity of lunar soils is typically only  $10^{-4}$  to  $10^{-5}$  g of adsorbate per g of soil, and the crystalline rock is probably a factor of 10 to 100 less adsorptive<sup>11</sup>. Assuming that the upper 1 mm of the entire lunar surface fully adsorbs gases with a capacity of  $10^{-5}$ , then the total capacity is  $5 \times 10^8$  kg. This is comparable to the estimated minimum mass needed to produce a long-lived atmosphere. Consequently, surface adsorption of gases would at worst result in a slight delay in atmospheric growth but would not act as a permanent barrier.

The significance of these quantities can be evaluated by examining ways of artificially adding gases to the lunar atmosphere. Each Apollo mission deposits nearly  $10^4$  kg of rocket exhaust into the lunar environment<sup>4</sup>. Since this is much less than the  $10^8$  kg needed to create a long lived atmosphere, exploration at the rate of several Apollo missions per year would seem to present no lasting hazard to the lunar environment. A permanent lunar base would possibly release gas at a rate equivalent to  $10^{-2}$  kg  $s^{-1}$  per man, assuming supply traffic equal to one Apollo mission month<sup>-1</sup> per man. Therefore small lunar colonies would probably not produce a long lived atmosphere. But even modest lunar exploration is likely to release gas faster than the present natural source rate of  $\sim 20$  g  $s^{-1}$ , resulting in a lunar atmosphere in which the gases of natural origin would be only trace components. Vigorous lunar exploration and colonisation could easily result in gas release rates greater than  $10^{-2}$  kg  $s^{-1}$  per man. Proposed activities such as placing factories on the Moon to avoid pollution of the Earth's atmosphere<sup>14</sup> need careful evaluation to avoid lasting contamination of the lunar environment.

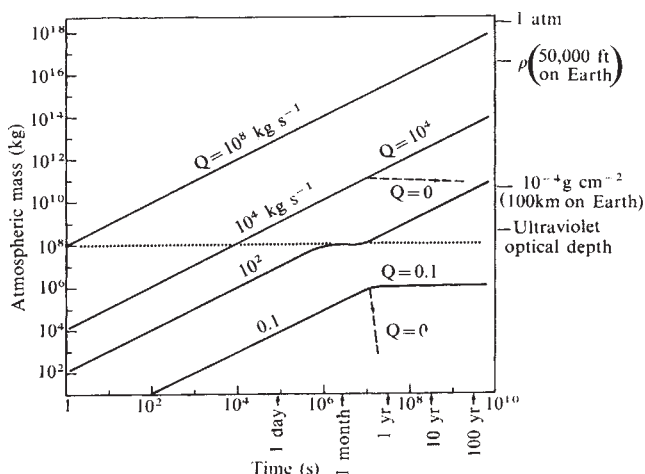


FIG. 2 Growth curves of the lunar atmosphere for various constant gas addition rates,  $Q$ . The transition to a long lived atmosphere occurs at a total mass of  $10^8$  kg which requires a rate of release of gas of about  $10^2$  kg  $s^{-1}$ . The present lunar atmospheric mass is approximately  $10^4$  kg and is maintained by a natural source of  $\sim 20$  g  $s^{-1}$ . Comparable densities in the terrestrial atmosphere are indicated. ---, Decay in total mass if the gas source is shut off



If one wanted intentionally to create an artificial lunar atmosphere, gases can be obtained by heating or vaporisation of the lunar soil. Approximately 25 MW is needed to produce  $1 \text{ kg s}^{-1}$  of oxygen by soil vaporisation. An efficient mechanism for gas generation is subsurface mining with nuclear explosives. K. Ehrlicke (in a North American Rockwell report) estimates that a 1 kton nuclear device will form a cavern approximately 40 m in diameter from which  $10^7 \text{ kg}$  of oxygen can be recovered. Application of this technique can easily generate enough gas ( $10^8 \text{ kg}$ ) to drive the Moon into the long lived atmosphere state.

The desirability of intentionally increasing the density of the lunar atmosphere is highly questionable, since the primary applications of a lunar laboratory involve utilisation of the present lunar 'vacuum'<sup>15-17</sup>. But the artificial generation of an atmosphere can be considered as another potential method for human modification of planetary environments<sup>18-21</sup>.

I thank members of the Rice University Department of Space Physics and Astronomy and R. B. Cathcart for discussions. This research was supported by NASA.

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Received October 12, 1973.

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for ground-based observations<sup>7</sup>, we have measured the relative thermal emission from the rings and disk of Saturn in the band from 29 to  $43 \mu\text{m}$ . This measurement provides an important constraint for separating the thermal properties of Saturn from those of its rings.

Ground-based photometry extending to  $\sim 40 \mu\text{m}$  is possible from high altitude sites under dry weather conditions<sup>7,8</sup>. The measurements between 29 and  $43 \mu\text{m}$  reported here were made with the 224-cm telescope at Mauna Kea Observatory in March 1973. Additional measurements at 17 to  $28 \mu\text{m}$  for normalisation purposes were recorded in September 1973. For convenience we refer to the band 29 to  $43 \mu\text{m}$  (FWHM) as the 34- $\mu\text{m}$  band, and the band 17 to  $28 \mu\text{m}$  as the 20- $\mu\text{m}$  band, although the effective wavelengths may depart significantly from these nominal values depending upon the source spectrum. In both spectral bands the ring and disk components were measured separately with a 9 arc s aperture centred on the disk and on the ansae at Cassini's division (the latter position subtended about equal areas of the A and B rings). The beam was sinusoidally chopped over a distance of 27 arc s along a direction aligned within a few degrees of Saturn's polar axis. East and West ansa readings did not differ significantly and were averaged in the final result. A check of scattered disk radiation at a polar sky position 18 arc s from the disk centre gave no measurable reading.

The ratio of the signals obtained for the ring and disk positions was  $0.39 \pm 0.05$  in the 34  $\mu\text{m}$  band and  $0.49 \pm 0.02$

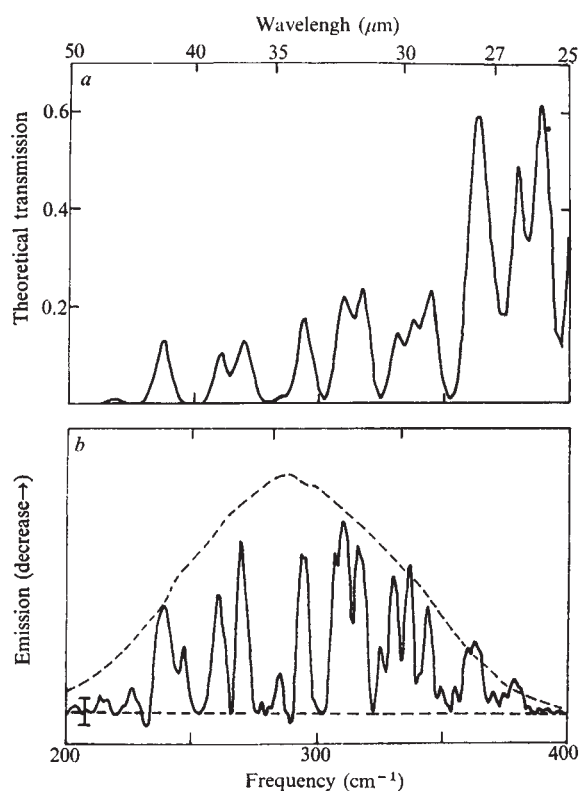


Fig. 1 Measured sky emission, compared with theoretical atmosphere transmission. *b*, Sky emission spectrum ( $\sim 2 \text{ cm}^{-1}$  resolution) measured with the 34- $\mu\text{m}$  filter and detector system attached to an interferometer. The saturated portions of the sky emission spectrum were used to establish the dashed baseline. The dashed line spectrum is computed from laboratory blackbody measurements, at a lower resolution, and shows the emission which would be observed on the same scale from a 270 K blackbody with an emissivity of 0.2 and the same filter system. *a*, Corresponding spectral features in a theoretical atmosphere transmission spectrum which has been computed by V. Kunde for 0.5 mm of atmospheric water content and a spectral resolution of  $5 \text{ cm}^{-1}$ .

## Thermal emission of Saturn's rings and disk at 34 $\mu\text{m}$

OBSERVATIONS from aircraft of Saturn's thermal emission do not resolve the flux component of the rings from that of the planet<sup>1,2</sup>. Ground-based observations in the windows at 11 and  $20 \mu\text{m}$ , which resolve the rings and disk contributions, include only a small fraction of the thermal flux and are sensitive to greenhouse effects<sup>3-6</sup>. Using new filter techniques

in the 20  $\mu\text{m}$  band, where the errors represent one standard deviation of the mean. To correct the 34- $\mu\text{m}$  measurement for instrumental effects, we compared the 20- $\mu\text{m}$  result to the transmission-weighted spectral ratio for the rings and disk of  $0.82 \pm 0.02$  determined from Murphy's 20- $\mu\text{m}$  band brightness temperatures<sup>6</sup>. We assumed 1 mm of precipitable water vapour for determining the transmission-weighting function. This value for the 20- $\mu\text{m}$  observations was inferred from daytime water vapour observations. The difference between the present ratio 0.49 and 0.82 from Murphy's data gives the composite correction for aperture filling factors (geometrically  $\sim 0.7$  for the ring position), diffraction of ring flux out of the aperture and different source 'dwell times' with our sinusoidal chopping for the ring and disk readings. From this 20- $\mu\text{m}$  calibration procedure, we deduce that the transmission-weighted specific intensity ratio of the rings (A and B) to the disk is  $0.67 \pm 0.13$  in the 34- $\mu\text{m}$  band. This allows for an additional 3% diffraction loss computed for the ring signal at this longer wavelength. We have assumed that systematic errors due to centering differences are small.

The interpretation of our results does not depend on knowing the absolute transmission and depends on the relative transmission spectrum only in so far as the ring and disk spectra differ. Because of the collision-induced  $\text{H}_2$  opacity source at 28  $\mu\text{m}$ , model atmosphere calculations depart from that of a blackbody in this spectral region<sup>9</sup>. Figure 1 compares a zenith sky emission spectrum, obtained during dry atmospheric conditions in March, with the theoretical transmission for 0.5 mm precipitable water vapour content. We divided the sky emission spectrum of Fig. 1b, which also incorporates the filter transmission properties, by a 270 K Planck spectrum. This result approximates the relative atmospheric-system transmission for weighting model spectra of the rings and disk. Saturn was observed at 34  $\mu\text{m}$  through less than 1.2 air masses and with atmospheric conditions comparable to those for the spectrum shown in Fig. 1b.

We can use the 34- $\mu\text{m}$  observations to derive a model effective temperature for the planet by assuming: (i) that the A and B rings exhibit the same average brightness temperature of  $91 \pm 3$  K in the 34- $\mu\text{m}$  band as in the 20- $\mu\text{m}$  band<sup>6</sup>; and (ii) that Trafton's model atmosphere calculations represent the planetary spectrum<sup>9</sup>. We obtain a model effective temperature of  $103 \pm 6$  K by an extrapolation of Trafton's spectrum for a 100 K model with zero helium abundance. This result compares to a blackbody brightness temperature of  $99 \pm 6$  K for the planet in our 34- $\mu\text{m}$  bandpass. Present models of the ring involving large particles lend some support to an assumption of a fairly constant ring brightness temperature between 17 and 43  $\mu\text{m}$  (ref. 10). The lower ring temperature of 83 K observed at 12  $\mu\text{m}$  in 1969 (ref. 4) can be explained by assuming the ring brightness temperature increases as the Saturnicentric declination of the sun increases<sup>6</sup>.

We can also use aircraft measurements of the total system flux to estimate ring and disk temperatures. Armstrong *et al.*<sup>1</sup> report a composite brightness temperature of  $89 \pm 4$  K in the band 30 to 45  $\mu\text{m}$ . Their quoted error does not allow for the uncertainty in absolute flux calibration. The resolution of this measurement into ring and disk components, consistent with our measurement of their relative emission in approximately the same bandpass, yields an average blackbody brightness temperature of  $\sim 86$  K for the rings and a model effective temperature of  $\sim 96$  K for the planet. These temperatures do not differ significantly from those derived above if we allow for the absolute flux calibration uncertainty in the aircraft measurement and differences in bandpass characteristics.

The results reported here support previous suggestions of a large internal heat source for Saturn<sup>2</sup>. For example, using Walker's bolometric albedo<sup>11</sup> of 0.61, we calculate a solar equilibrium temperature of 71 K. A model effective temperature

of 96 to 103 K implies an internal heat source of 2.3 to 3.4 times the solar insolation.

We acknowledge discussions with Professor W. M. Sinton, and Drs L. M. Trafton, V. Kunde, and L. J. Caroff; and observational assistance by Messrs D. F. Lester, M. W. Cromar, and the Mauna Kea staff. This research was supported by grants from the Air Force Office of Scientific Research and the National Aeronautics and Space Administration. I.G.N., J.V.R. and H.C.F. are guest observers at the Mauna Kea Observatory, Institute for Astronomy, University of Hawaii.

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## Reflection from a transversely moving mirror

ALTHOUGH there have been many experiments which demonstrate the first order Doppler effect in the reflection of electromagnetic waves from a mirror with a component of radial motion, there has not yet been a precise determination of the frequency of light reflected from a transversely moving mirror. There is some confusion about whether light reflected from a mirror moving transversely to an incident beam should be reflected at a frequency which is different from that of the beam, because of a relativistic (second order Doppler) effect<sup>1,2</sup>. Any frequency shift would be extremely small, and there are severe experimental difficulties in attempting to observe a shift of magnitude equal to or less than that associated with the transverse Doppler effect. The experiment described here was designed to overcome these difficulties and to investigate the possibility of frequency shifts of a smaller magnitude than that which would correspond to the second order Doppler shifts associated with transversely moving emitters<sup>3</sup>. The overall accuracy of the experiment is better than one part in  $10^{16}$ .

A rotating mirror, supported by an air bearing, is in one arm of a Michelson interferometer. The other arm has a



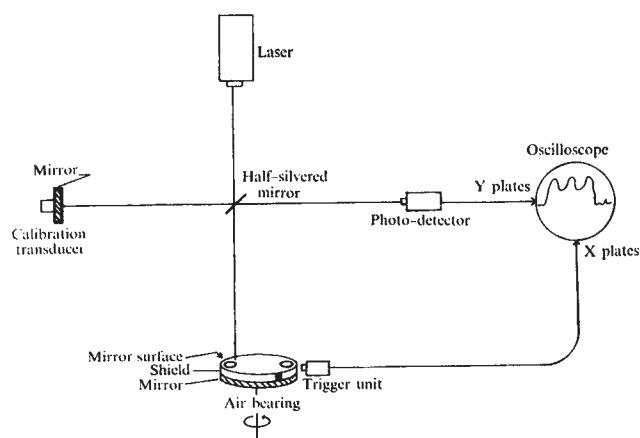


FIG. 1 The arrangement for synchronising the display from a Michelson interferometer in order to investigate any frequency shift in light reflected from a transversely moving surface.

mirror which can be moved in the direction of the reference light beam by a crystal transducer. This mirror, when moved, demonstrates that the equipment is functioning correctly, by changing the position of the fringes displayed on the oscilloscope screen.

The fringes are monitored by the photodetector, which delivers an output to the 'Y' plates of an oscilloscope. The oscilloscope time-base is triggered at the same frequency as the rotation of the mirror. Thus each particular point on the mirror corresponds to one particular part along the 'X' axis of the display. This removes the effects of variations in the mirror profile and of periodic vibrations of the rotating mirror which are related to the frequency of rotation.

Any relativistic frequency shift produced by the transverse motion should cause the two beams to beat together and thus the pattern displayed on the oscilloscope should undulate at each value of X. The control mirror, which may be considered as introducing a first order Doppler effect, shows this effect quite clearly. Calculations show that at the transverse speeds of  $5\text{--}20\text{ m s}^{-1}$  used in the experiment, beat frequencies of the order of one beat per second to one beat in twelve seconds would be produced by a mechanism of the magnitude of the second order Doppler effect for a transversely moving emitter. Observation for 10 times the associated beat period showed no evidence of any shift in the position of the fringes in the display. The apparatus is being modified and we hope to improve the accuracy of the experiment and then to publish the results in greater detail.

An explanation of why no frequency shift occurs has been produced by using standard transforms of direction cosines of electromagnetic waves. The light is received at the mirror surface at an angle of approach and shifted to the blue. The blue light is then emitted specularly at the mirror, making an equal and opposite angle to the normal to the mirror surface. This reflected beam is received back in the laboratory at normal incidence and restored once more to its original colour. Einstein's equation<sup>4</sup> is a restricted analysis of the complete phenomenon. It is restricted to motions in the direction of the normal to the mirror surface. Reflection by a transversely moving mirror is the other special case.

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<sup>4</sup> Einstein, A., *Annln Phys.*, **17**, (1905).

## Discovery of three earthquake faults in Iran

AN earthquake fault is a ground fracture formed in association with a shallow earthquake, usually by reactivation of a pre-existing geological fault. It can provide information about both the mechanism of seismic energy release during an earthquake and the accompanying regional strains. Until now only two such faults which have been adequately documented are known in Iran. These are associated with the Buyin Zara (1962) and the Dasht-e Bayaz (1968) earthquakes<sup>1-3</sup>. The Torud (1953) and the Ashkhabad (1948) earthquake<sup>4,5</sup> (the latter in the USSR, just north of the Iranian frontier), were probably also accompanied by faulting, but complete documentation is lacking. Here, we describe three additional earthquake faults found during recent seismotectonic field studies in Iran. The earthquakes involved were at Selakhor (1909), Baghan-Germab (1929) and Salmas (1930). Figure 1 shows the location of the three faults and of the other known and probable faults already mentioned.

The Selakhor earthquake occurred at 02 h 48 min 18 s GMT on January 23, 1909 in the upper Ab-i Diz valley in the Selakhor District of the Zagros mountains (south-western Iran). The body wave magnitude of the event was 7.4 (ref. 6). Maximum destruction was confined to the south-eastern part of the valley, between the village of Zargina and the small town of Dorud, with the preliminary macro-seismic epicentre (taken as the centre of the area of maximum destruction) located at about  $33.5^{\circ}\text{N } 49.0^{\circ}\text{E}$  (Fig. 2).

Contemporary descriptions of ground deformation<sup>7</sup> suggest that faulting occurred during the earthquake. The eroded fault scarp is clearly visible now on the ground and on aerial photographs, and can be followed in a  $135^{\circ}$  direction from Kalangona to Dorud and Sarawand (Fig. 2). In the alluvium of the Ab-i Diz Valley it appears as a rectilinear



FIG. 1 Location of known earthquake faults in Iran. Dates indicate year of earthquake associated with faulting, with double frame for the three faults studied here. Question mark for cases in which documentation is incomplete.

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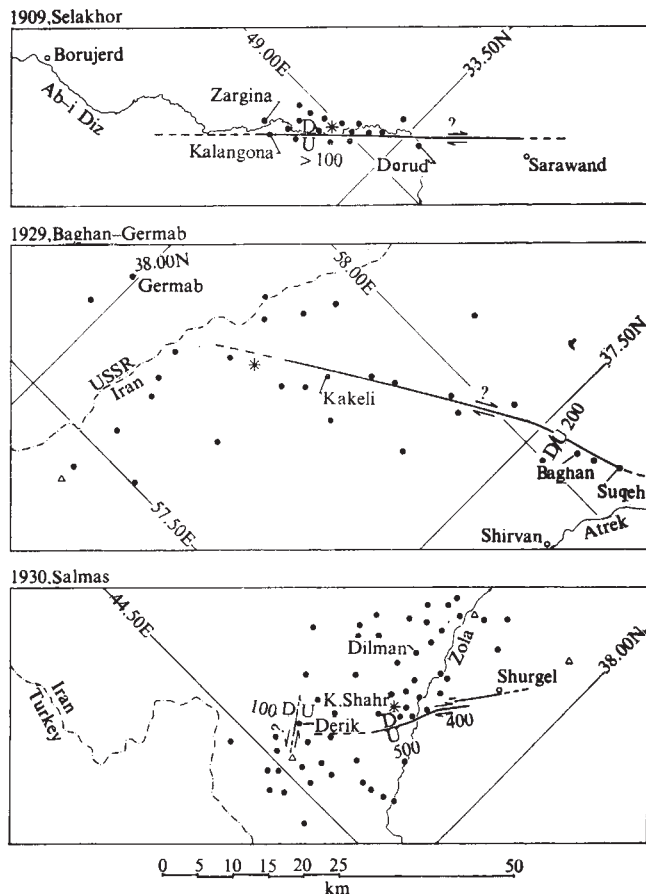


FIG. 2 Earthquake fault trace and damage distribution. Black line indicates fault trace, dashed where uncertain. Vertical fault displacements during the earthquake are shown by U (up) and D (down) and horizontal displacements by double arrow. Numbers are displacements in cm, with question mark where sense of displacement is known but not the amount. ●, Villages totally destroyed or very severely damaged. \*, Approximate centre of area of maximum destruction, taken as macroseismic epicentre. △, New thermal spring created, or existing spring modified, by the earthquake.

topographical step along which the north-eastern side is downthrown by at least 1 m. Horizontal displacements have so far not been determined. South-east of Dorud, the earthquake fault enters the mountain range where it can be seen to coincide with a well marked pre-existing geological fault. The total length of the 1909 fault scarp exceeds 40 km.

Detailed geological mapping has shown that the earthquake fault was formed by reactivation of a much larger NW-SE structure, the Main Recent Fault. This is a major wrench fault extending over 1,700 km from eastern Anatolia to the Gulf of Oman<sup>8</sup>. It is later than, and quite distinct from, the post-Pliocene Main Thrust<sup>9,10</sup> which is considered to mark the northeastern edge of the Arabian Plate<sup>11</sup> (Fig. 1). The Main Recent Fault probably results from the latest NNE motion of the Arabian Plate with respect to Central Iran, and is at present seismic to the north-west of the 1909 epicentral region, but in the south-east no data has yet been found to establish its activity.

The Baghan-Germab earthquake occurred at 15 h 37 min 36 s GMT on May 1, 1929 in the Kopet Dagh mountains (north-eastern Iran) with a magnitude of 7.1 (ref. 6). Maximum destruction and ground deformation were observed along a NNW-SSE zone between Baghan (Iran) and Germab (USSR), with the preliminary macroseismic epicentre located at 37.8°N 57.8°E (Fig. 2).

Surface faulting over a length of about 50 km was associated with the earthquake, extending across the fold

trend in a 150° direction from Suqeh to the vicinity of the Soviet border (Fig. 2). It was mapped along two sections, at Baghan and at Kakeli, and the overall trace was drawn on the basis of aerial photographs together with information from contemporary accounts<sup>12</sup> and interviews with survivors of the catastrophe. Today, the eroded scarp is in places up to 2 m high, with the north-eastern side uplifted (Baghan region). There is also some indication of dextral strike-slip movement, but more field work is required to obtain accurate measurements of this displacement. The linearity of the earthquake fault through varying topography indicates that it is probably vertical at depth.

The 1929 earthquake fault resulted from reactivation of a post-Pliocene fault zone which extends from Bakharden (USSR) to Quchan (Iran) (Fig. 1). This zone forms part of a fault system which dissects the late Alpine folds of the Kopet Dagh and consists of NNW dextral and NE sinistral strike-slip faults and minor E-W thrusts. This system is consistent with a NNE-SSW direction of tectonic compression. Quaternary horizontal displacements on some of the individual faults amount to several kilometres<sup>13</sup>, and several have been shown to be active<sup>14</sup>. In the north, the Bakharden-Quchan fault zone bends into the Main (Kopet Dagh) Fault which marks the southern edge of the Turan Plate. In the south the fault zone crosses the Atrek River near Quchan, which has been devastated at least three times by earthquakes during the 19th century.

The Salmas earthquake occurred in west Azarbaijan (north-western Iran) on May 6, 1930, almost exactly one year after the Baghan-Germab earthquake. It was preceded on the same morning by a moderately strong foreshock. The main shock, which occurred at 22 h 34 min 27 s GMT, had a magnitude of 7.2 (ref. 6) and caused extensive destruction throughout the Salmas valley and the mountains in the south and west near the Turkish frontier (Fig. 2). The preliminary macroseismic epicentre was located at about 38.2°N 44.7°E.

The earthquake fault scarp can be seen over about 20 km between Shurgel and the region west of Kohneh Shahr at the southern edge of the Salmas valley (Fig. 2). Its average direction is 120° and maximum displacements during the earthquake were about 4 m dextral and 5 m



FIG. 3 Salmas earthquake, 1930: Fault scarp south of Kohneh Shahr. The scarp crosses an early Christian cemetery (from bottom right of photograph to background). The ground, which was originally level, dropped by a maximum of 5 m north of the fault (left of photo). The two figures at foot of scarp provide a scale.



downwards on the north-eastern side (Fig. 3). Survivors of the earthquake describe the fault as extending another 10 km to the north-west, but no trace of this scarp can now be seen. At Derik, a separate earthquake fault downthrown about 1 m to the north-west extends for about 3 km through metamorphic rock in a 55° direction. The pattern formed by the location of Quaternary and active travertine springs suggests that the corresponding geological fault has a sinistral horizontal component. At the south-western end of the Derik fault a thermal spring ceased at the moment of the earthquake, and a new one appeared about 1 km further south-west in the same alignment. Other thermal springs appeared, and an existing one at the south-eastern end of the main fault segment increased its flow.

The sense of vertical displacement along the main earthquake fault, and observations of waterlogging in the fields of the Salmas valley, show that the valley subsided during the earthquake. The importance of the dextral displacements, however, and the movement along the Derik fault, indicate a more complex tectonic situation which may be interpreted in terms of a N-S or a NNE-SSW compression. The structure of this part of the country, which is now being mapped by the Geological Survey of Iran, has not yet been studied and it can only be noted at this stage that the general NW direction of the earthquake fault is also that of the major regional active faults: one passing through Tabriz and Maku in the north, the Main Recent Fault (Zagros) in the south, and the North Anatolian Fault further west, in Turkey.

The small number of earthquake faults known in Iran is surprising in view of the high seismicity of the country and the presence of faults known to be active<sup>15</sup>. This is possibly because aseismic fault activity (fault creep) plays a large part in the process of tectonic strain release. There is also a lack of adequate macroseismic documentation. In this respect it is interesting to note that four out of the five earthquake faults known with certainty (Fig. 1) were over 40 km long, and that all were associated with events of magnitude greater than seven. It seems probable that, as in other seismic countries<sup>16,17</sup>, shorter surface breaks have occurred in Iran during earthquakes of smaller magnitudes, but that these earthquake faults have passed unreported or even, in desert areas, unnoticed. The three earthquakes summarised here emphasise the danger, common in Middle Eastern seismotectonic research, of drawing conclusions on the only available basis of instrumental epicentre data covering the last one or two decades. With such data the regions of the 1909, 1929 and 1930 earthquakes seem to be relatively quiescent, and the activity of the associated faults is not noticed.

It would be premature at this stage to integrate the new information presented here with existing knowledge on the tectonics of Iran. The common characteristic of the three earthquake faults should, however, be kept in mind during future field studies. In all three cases, fault directions and displacements are most easily interpreted as indicating present day NNE-SSW compression. This manifests itself in the regions of contact between Central Iran and the adjoining plates—the Arabian Plate in the south-west (1909 earthquake) and the Turan Plate in the north-east (1929 earthquake), as well as in the slightly more internal region of West Azarbaijan (1930 earthquake). A predominantly NNE direction of plate motion has been postulated for Arabia on the basis of fault plane solutions for recent earthquakes<sup>18</sup>, but field evidence is so far lacking. It remains to be seen whether shallow earthquakes in more central parts of Iran may be similarly interpreted.

Detailed reports are in preparation by the authors for the 1909 earthquake (J. S. T., J. B.), the 1929 earthquake (J. S. T.) and the 1930 earthquake (J. S. T., M. B.). We thank N. Khadem, Managing Director of the Geological

Survey of Iran. A grant from the Natural Environment Research Council supported part of the work.

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Received October 23, 1973; revised January 23, 1974.

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## Geology and volcanic history of Pico Island Volcano, Azores

THE Azores Islands formed as a result of volcanism along a conservative plate margin<sup>1</sup>. The volcanism and seismicity of the archipelago have been described<sup>2</sup> together with chemical analyses of lavas from many of the islands<sup>3</sup>.

The Mid-Atlantic ridge crest isolates the most western islands, Flores and Corvo, from the rest of the archipelago. The semismicity of the ridge branches eastwards along a more subdued ridge; the Azores-Gibraltar-Rise. The latter is primarily a strike-slip fault zone with tensional characteristics and associated volcanism in the Azores region, and a compressional zone in the region immediately west of the Straits of Gibraltar<sup>4,5,6</sup>.

A secondary spreading ridge has formed in the Azores region, resulting from a combination of a different spreading rate north and south of the archipelago and a change in crustal spreading direction<sup>7</sup>. The secondary spreading centre is a graben known as the Terceira Rift<sup>8</sup>, within which three of the major Azorean volcanoes (Graciosa, Terceira and S. Miguel) have formed.

The earliest volcanism occurred in the extreme east of the island group, where basalt lava flows are exposed beneath limestones palaeontologically dated as Middle Miocene<sup>9</sup>. Potassium-argon dates have not yielded ages greater than 4 m.y. for lavas on Santa Maria Island and on the

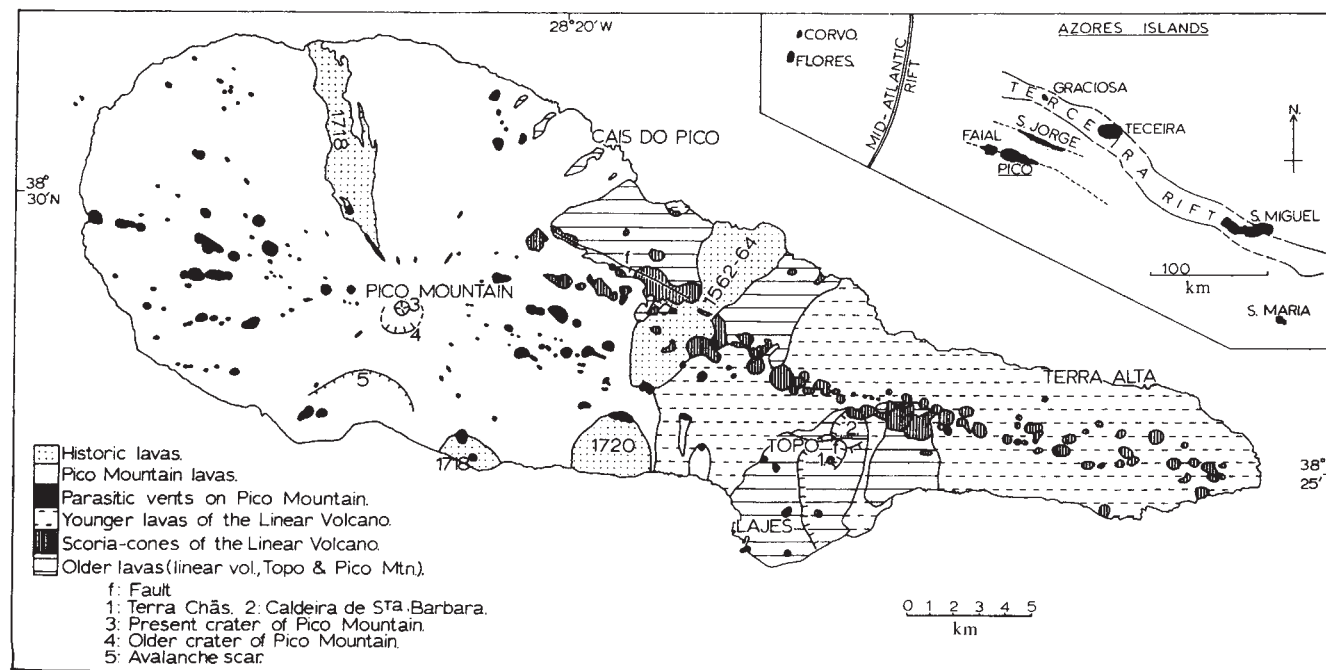


FIG. 1 Geological map of Pico Island Volcano.

extreme east of Sao Miguel<sup>10,11</sup>. The latest volcanism occurred in the islands of the central group, of which Pico is a member.

Pico Island seems to be the youngest island in the Azores and is covered by many recent basaltic lava flows. Eruptions were recorded in 1562–64 and 1718–20. The island has been mapped by the Portuguese Geological Survey<sup>12</sup>.

There is a central volcano, Pico Mountain (2,351 m), in the west and a linear volcano (1,076 m) dominates the eastern region (Fig. 1). The island is 46 km long and has a maximum width of 16 km. The volcanoes rise 3,500 m from the sea-floor and lie on a linear submarine ridge trending parallel to the Terceira Rift (Fig. 1, inset), which also includes the adjacent island of Faial.

The flanks of Pico Mountain are covered with recent lava flows erupted from radially aligned parasitic fissures. The latest activity comprises flank eruptions rather than eruptions from the central cone. The cone rises steeply above an altitude of 1,200 m with slopes up to 40°. Below this altitude, however, the slopes are seldom steeper than 8–10°. Probable gravity sliding on the steep flanks of the mountain has created a large avalanche scar (Fig. 1) on the south slopes. The slopes of Pico Mountain are composed of pahoehoe lavas derived directly from the summit crater (500 m diameter), which has since become filled almost completely by similar lavas. Fumaroles with temperatures of up to 60° C occur on the flanks of a lava cone which has formed in the north-eastern region of the crater.

The construction of the steep sided cone of Pico Mountain has probably resulted from repeated eruption, at very low rates of effusion, of pahoehoe lavas with little associated tephra, giving rise to highly compound lavas such as those exposed in the summit crater and at the foot of the mountain<sup>13</sup>. The flank lavas include both pahoehoe and aa flows.

The lavas of Pico Mountain contain phenocrysts of olvine and augite, in some cases with plagioclase. Crystal settling has occurred in many pahoehoe flows of ankaramiti nature, involving the accumulation of large olivine and augite phenocrysts in the lower part of a flow. Radiating plagioclase glomero-groups characterise the pahoehoe lavas of the steep sided cone. The historic Santa Luzia flow (1718), north-west of the mountain, (Fig. 1) also contains phenocrysts of hornblende, magnetite and apatite.

The Linear Volcano is offset to the north relative to Pico

Mountain. It has evolved by repeated fissure eruptions along WNW trending fissures. Young lava flows occur along the entire 30 km length, except in the extreme north-west.

The Topo Volcano is situated halfway along the Linear Volcano, slightly to the south of its main axis (Fig. 1). It is controlled by NE–SW trending fissures. Evidence suggests, however, that it has evolved as a volcano transitional between a linear and a central volcano. It is surrounded by the younger lavas of the Linear Volcano and it conveniently divides the latter into a western and an eastern Linear Volcano. No young lavas occur on the Topo Volcano.

The western Linear Volcano is divided equally into an older north-western region characterised by eroded scoria-cones together with recent fault scarps, and an eastern region dominated by young scoria-cones situated along a narrow fissure zone (Fig. 1).

The eastern Linear Volcano is also dominated by young scoria-cones, but older cones, partially buried by the younger lava flows, also occur. High cliffs which are mostly degraded,

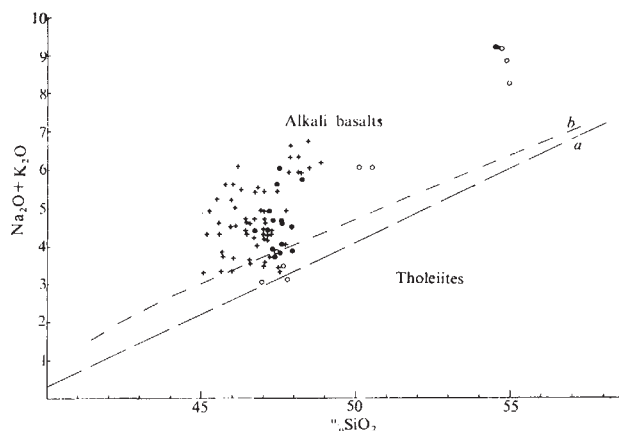


FIG. 2 Alkalies: silica diagram for Pico rocks: ●, lavas of Pico Mountain central volcano; ○, lavas of 1718 and 1720 (Pico Mountain volcano); +, lavas of the Linear Volcano and Topo. Dashed lines divide alkali basalts from tholeiites: a, from Macdonald (1968); b, from Irvine and Baragar (1971).



such as Terra Alta, occur on both the north and south sides of this narrowest zone of the island (Fig. 1).

The younger lavas of the Linear Volcano seem to be contemporaneous with those of Pico Mountain.

The Topo Volcano had an old summit crater which subsequently became filled in by thick pahoehoe lavas. These may have formed a lava lake. The original crater region has been modified by caldera collapse of the 'trap door' type<sup>14</sup> with arcuate scraps only partially enclosing a caldera, for example, the Terras Chas Depression and the Caldeira de Santa Barbara (Fig. 1). Parasitic vents and fault scraps occur to the south-west of the collapse region, and thin dykes together with some buried scoria-cones are exposed in the cliffs along the south coast.

Early in its history the Topo Volcano probably resembled the shield volcanoes of the western Galapagos Islands<sup>15</sup> but the morphology has been modified by the caldera collapse. Only four lavas seem to postdate this event. The oldest lavas of the adjacent Linear Volcano postdate all but the latest Topo lavas.

The oldest lavas on Pico occur in the north-western region of the Linear Volcano, and on the Topo Volcano, and in both cases the lava flows are covered by ash and/or soil. Scoria-cones are considerably eroded. The oldest lavas are exposed at the foot of the high cliffs along the south coast of the Topo Volcano. Similarities between the two areas suggest that they are probably of the same age.

The lavas of the Linear Volcano are dominantly aa flows. There are very few pahoehoe lavas, although an extensive flow was erupted in the period 1562–64, from the older, western region (Fig. 1). The majority of the lavas contain phenocrysts of olivine, augite and plagioclase but older lavas may contain only olivine phenocrysts. Many of the Topo lavas are similar to those of Pico Mountain, and spectacular crystal settling, involving the accumulation of olivine and augite phenocrysts, characterises many of the pahoehoe lavas. This phenomenon is also present in the thick pahoehoe lavas which fill the old, pre-caldera, summit crater referred to already. Less porphyritic aa flows are common together with aphyric lava flows which are more commonly associated with the Topo Volcano. There are also plagioclase rich basalts which resemble those on the steep sided cone of Pico Mountain. Biotite occurs as a rare accessory mineral in some Topo lavas.

Figure 2 indicates the alkaline nature of the analysed lavas of Pico. Some hypersthene normative lavas occur and they plot on the boundary between tholeiites and alkali basalts<sup>16,17</sup>. The most highly differentiated lava is a single flow of benmoreite erupted on the northwestern flanks of Pico Mountain in 1718. This is part of a lava series erupted in 1718 and 1720, which ranges in composition from olivine basalt (hypersthene normative) to hawaiite and then to benmoreite.

Few differentiated lavas occur on Pico Mountain, and there is only very slight differentiation in the older lavas of the Linear Volcano and Topo. The younger lavas (Fig. 1) of the Linear Volcano are interpreted as a differentiated series akin to that mantling the older 'oceanite' series on Reunion Island (Indian Ocean)<sup>18</sup>. The Pico differentiated series ranges from plagioclase basalts to hawaiite and includes two flows transitional to mugearite.

The author acknowledges the receipt of a Natural Environment Research Council studentship for 1970–73. The help given by Dr Victor-Hugo Forjaz of the University of Lisbon is also acknowledged along with the supervision given by Dr T. W. Bloxam and Dr P. R. Hooper.

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Received November 26, 1973; revised January 29, 1974.

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## Heteromolecular clusters of H<sub>2</sub>O, SO<sub>2</sub>, CO<sub>2</sub>, CO and NO

HOMOGENEOUS clusters of gas molecules in isentropically expanding jets, first observed in 1961<sup>1,2</sup>, have been the subject of several experimental studies<sup>3–7</sup>. Mixed or heteromolecular clusters have also been observed in mixtures of two or more gases expanded from a high pressure reservoir. Leckenby *et al.*<sup>8</sup> have identified the species Ar<sub>2</sub>·CO<sub>2</sub>, Ar·(CO<sub>2</sub>)<sub>2</sub> and larger clusters, and N<sub>2</sub>·O<sub>2</sub>, SF<sub>6</sub>(CO<sub>2</sub>)<sub>2</sub> and SF<sub>6</sub>·CO<sub>2</sub>·Ar. Milne *et al.*<sup>9</sup> have observed mixed H<sub>2</sub>O–Ar clusters and the heteromolecular dimers O<sub>2</sub>·H<sub>2</sub>O and NO·H<sub>2</sub>O.

We observed mixed clusters of the type SO<sub>2</sub>·(H<sub>2</sub>O)<sub>n</sub>, *n* = 1–6, of the same order of magnitude as pure water clusters when 2 mmHg of SO<sub>2</sub> were added to 1,500 mmHg nitrogen, saturated with water of room temperature, and expanded through an orifice of 0.0386 cm. The possible implications of the homogeneous formation of such hydrated cluster species to upper atmospheric chemistry<sup>10</sup> and aerosol formation<sup>11</sup>, led to a series of experiments with a water-saturated nitrogen carrier which was seeded with trace gas species

TABLE 1 Heteromolecular clusters observed in fully expanded jets\*

| Species                                                         | <i>n</i> | Concentration in nitrogen                    |
|-----------------------------------------------------------------|----------|----------------------------------------------|
| SO <sub>2</sub> (H <sub>2</sub> O) <sub>n</sub>                 | 1–5      | 0.5% SO <sub>2</sub>                         |
| CO <sub>2</sub> (H <sub>2</sub> O) <sub>n</sub>                 | 1–5      | 5% CO <sub>2</sub>                           |
| NO(H <sub>2</sub> O) <sub>n</sub>                               | 1–6      | 1% NO                                        |
| H <sub>2</sub> O(SO <sub>2</sub> ) <sub>n</sub>                 | 1, 2     | 0.5% SO <sub>2</sub>                         |
| (CO <sub>2</sub> ) <sub>2</sub> (H <sub>2</sub> O) <sub>n</sub> | 1, 2     | 5% CO <sub>2</sub>                           |
| (NO) <sub>2</sub> H <sub>2</sub> O                              | –        | 0.5% NO, 0.25% SO <sub>2</sub>               |
| NO·SO <sub>2</sub> ·(H <sub>2</sub> O) <sub>n</sub>             | 1–3      | 0.5% NO, 0.25% SO <sub>2</sub>               |
| CO <sub>2</sub> ·SO <sub>2</sub> ·H <sub>2</sub> O              | –        | 2.5% CO <sub>2</sub> , 0.25% SO <sub>2</sub> |
| NO(SO <sub>2</sub> ) <sub>n</sub>                               | 1, 2     | 0.5% NO, 0.15% SO <sub>2</sub>               |
| NO(CO <sub>2</sub> ) <sub>n</sub>                               | 1, 2     | 0.5% NO, 2.5% CO <sub>2</sub>                |
| CO <sub>2</sub> (NO) <sub>n</sub>                               | 1, 2     | 2.5% CO <sub>2</sub> , 0.5% NO               |
| SO <sub>2</sub> (CO <sub>2</sub> ) <sub>n</sub>                 | 1, 2     | 0.25% SO <sub>2</sub> , 2.5% CO <sub>2</sub> |
| CO(CO <sub>2</sub> ) <sub>n</sub>                               | 1–3      | 0.5% CO, 2.5% CO <sub>2</sub> †              |

\* Total source pressure, *P*<sub>0</sub> = 2,000 mmHg. Orifice diameter, *d* = 0.0343 cm. The first eight experiments were carried out with nitrogen saturated with water of room temperature. The remainder were conducted with dry nitrogen. The species N<sub>2</sub>·H<sub>2</sub>O, N<sub>2</sub>·SO<sub>2</sub>, N<sub>2</sub>·(SO<sub>2</sub>)<sub>2</sub>, N<sub>2</sub>·CO<sub>2</sub>, and N<sub>2</sub>·NO were also observed mainly as a result of the large amount of nitrogen present.

† Under the present conditions CO did not cluster appreciably with any species except CO<sub>2</sub>.

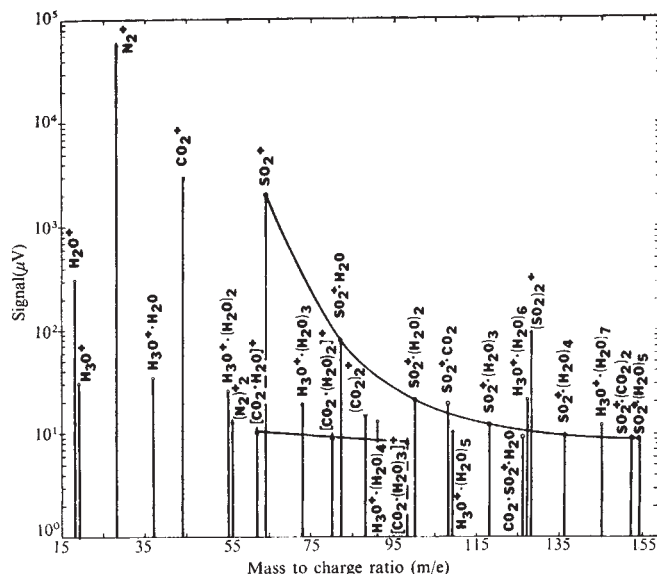


Fig. 1 Heteromolecular clusters observed in a fully expanded jet composed of 10%  $\text{H}_2\text{O}$ , 2.5%  $\text{CO}_2$  and 0.25%  $\text{SO}_2$  in nitrogen. The  $\text{SO}_2^+(\text{H}_2\text{O})_n$  and the  $[\text{CO}_2^+(\text{H}_2\text{O})_n]^+$  series are connected by curves for convenience.

which are known to exist in the atmosphere and in the exhausts of jet aircraft.

The supersonic molecular beam apparatus consists essentially of a three-stage differentially and cryogenically pumped vacuum system<sup>5,6</sup>. Gases and/or vapours are admitted through a sonic orifice at the end of a source probe, and the beam is formed by a helium-cooled skimmer (20° K) and then further collimated. Beam species were detected with a quadrupole mass spectrometer after mechanical beam chopping and subsequent phase-sensitive signal processing with a lock-in amplifier. For some of the latest experiments the mass spectrometer was controlled by a minicomputer and the ion signals were digitised.

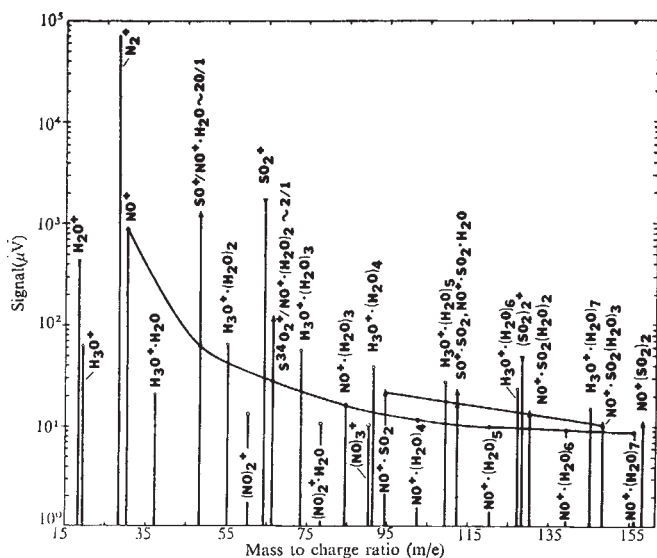
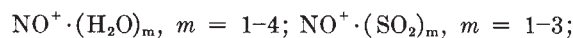


Fig. 2 Heteromolecular clusters observed in a fully expanded jet composed of 10%  $\text{H}_2\text{O}$ , 0.5%  $\text{NO}$  and 0.25%  $\text{SO}_2$  in nitrogen. The  $\text{NO}^+(\text{H}_2\text{O})_n$  and the  $\text{NO}^+\text{SO}_2^+(\text{H}_2\text{O})_n$  series are connected by curves for convenience. The  $\text{SO}_2^+(\text{H}_2\text{O})_n$  series was also present in this expansion but it is not shown here in order to simplify the spectrum. It is of approximately the same magnitude as in Fig. 1.

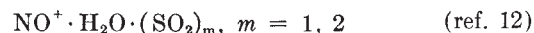
Table 1 summarises the experimental conditions and the mixed cluster species observed. Figures 1 and 2 present

sample mass spectra to give an indication of the relative magnitudes of the ion signals. The total source pressure and orifice diameter for both cases are 2,000 mmHg and 0.0343 cm respectively. Only those peaks directly related to the heteromolecular clusters are shown, with the water cluster ion series (that is,  $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ) included for comparison. The ionising electron energy was 90 V. The species actually detected were ions produced in the mass spectrometer ion source. The ionisation occurred, however, in the free molecular flow regime after all collisions had ceased and, therefore, the formation of the heteromolecular clusters was completely homogeneous. Ion-molecule reactions can be excluded because of the low pressure in the mass spectrometer ion source (about  $10^{-8}$  mmHg) and because the observed signals were in phase with the beam chopper. A more extensive analysis of the results will be published elsewhere.

Species similar to those in Table 1 have been heterogeneously generated by subjecting low pressure gas mixtures (1–4 mmHg) to an electrical discharge and allowing them to react in a field-free reaction cell<sup>12</sup>. In this manner the following cluster ions were observed mass spectrometrically:



and



Pending further investigation, we think that the observed mixed cluster signals are mostly virgin species formed through a simple gas kinetic mechanism, rather than from complex fragmentation of a condensed bulk phase on electron impact in the ion source of the mass spectrometer. Although the low temperatures attained in the nitrogen expansion are certainly sufficient to condense water, thus raising the possibility of some fragmentation of the bulk phase, there is some experimental<sup>13</sup> and theoretical<sup>14</sup> evidence to suggest that this is secondary to other effects. Also, in a recent experimental  $\text{CO}_2$  condensation study, massive fragmentation was presumed absent because of the agreement between the observed and predicted dependence of cluster concentrations on source pressure and orifice diameter<sup>15</sup>.

The formation of the heteromolecular dimer probably is the rate-controlling step in the growth of mixed clusters. The dimer must be formed by a termolecular collision and higher clusters then grow by the successive bimolecular addition of monomers. A simple gas kinetic model with termolecular dimer formation and bimolecular destruction seems to be adequate to predict dimer concentrations in expanding jets<sup>5,15</sup>. The surprising persistence of the species (Table 1), despite the relatively low concentrations of the apparent species in nitrogen, can only be explained by postulating strong affinity, with nitrogen serving as the third body in the dimer formation mechanism.

The results presented here have established the existence and identity of mixed clusters of  $\text{H}_2\text{O}$ ,  $\text{SO}_2$ ,  $\text{CO}_2$ ,  $\text{NO}$  and  $\text{CO}$  formed during homogeneous nucleation processes in an isentropic expansion from the gas phase. There are numerous observations of the homogeneous nucleation of water vapour which has been appreciably enhanced by the presence of foreign gas molecules<sup>16-18</sup>. Clathrate structures of water molecules around foreign gas molecules, particularly those found as trace contaminants in the atmosphere, and most notably, in the present context,  $\text{SO}_2$  and  $\text{CO}_2$  may be important in aerosol physics, and furthermore, favourable conditions for the production of water clathrates are known to be present in the exhausts of jet aircraft<sup>9</sup>.

The mass spectra data acquisition was performed principally by Mr A. B. Bailey, ARO Inc., Arnold Engineering Development Center, Tennessee. This work was jointly sponsored by the United States Air Force and the Climatic Impact



Assessment Program, Office of the Secretary, United States Department of Transportation.

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Received December 17, 1973.

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## Non-linearity of hydrogen bonds in molecular crystals

IN recent studies of the non-linearity of hydrogen bonds<sup>1,2</sup>, histograms have been shown of the O-H ··· O angles,  $\psi$ . The distributions reflect the energy dependence of the hydro-

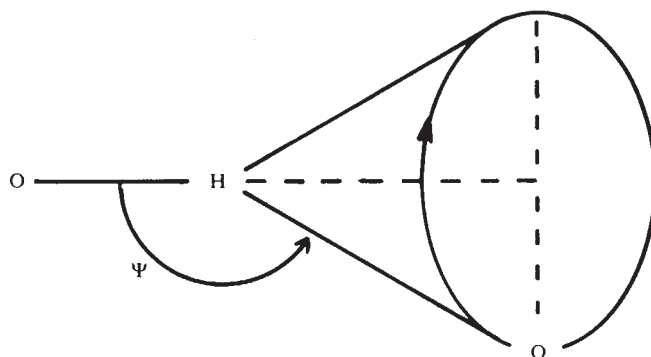


FIG. 1 Cone of opening angle ( $360^\circ - 2\psi$ ) illustrating the number of possible hydrogen-bond configurations with O-H ··· O angle,  $\psi$ .

gen bond on the angle  $\psi$ . In such distributions maxima are found at values of  $\psi$  approximately  $15^\circ$  from the linear configuration. Model calculations on water dimer interactions<sup>3</sup> indicate, however, that the configuration of minimum energy involves a linear hydrogen bond.

Before angle distributions of the hydrogen bond as observed in crystal structures, are compared with results of theoretical calculations on non-linearity in simple hydrogen bond models, experimentally determined hydrogen bond angle frequencies should be properly adjusted. This is because the sampling procedure includes a factor which complicates the correct interpretation of the histograms. This factor, which is of a geometric nature, seriously influences the statistics and has not been taken into account in other studies. This factor does not affect the observed hydrogen bond angle in any single case, but only affects the frequency distribution of the whole set of bond angles.

Assuming that the optimum hydrogen bond is linear this factor depends on  $\psi$ , because the number of possible hydrogen bond configurations at any value of  $\psi$  is proportional to  $\sin \psi$ . This is shown in Fig. 1, which illustrates that the acceptor oxygen atom at a certain O-H ··· O angle,  $\psi$ , can select any of the positions that lie on the generator of a cone of opening angle  $360^\circ - 2\psi$ , with the hydrogen atom at the apex and the cone axis in line with the O-H bond.

Thus although the assumption that the energy depends on  $\psi$  indicates that the probability of a hydrogen bond with angle  $\psi$  decreases as  $\psi$  increases, in fact the probability will increase with increasing  $\psi$  because of the increase in the number of possible hydrogen bond configurations with angle  $\psi$ .

TABLE 1 Crystal structures selected for the hydrogen bond study

| Compound                                               | Ref. | Compound                                          | Ref. |
|--------------------------------------------------------|------|---------------------------------------------------|------|
| Allitol                                                | 4    | $\gamma$ -D-gulonolactone                         | 27   |
| Methyl $\alpha$ -D-altropyranoside                     | 5    | D-iditol                                          | 28   |
| DL-arabinitol                                          | 6    | Inosine                                           | 29   |
| $\beta$ -DL-arabinose                                  | 7    | Epi-inositol                                      | 30   |
| L-ascorbic acid                                        | 8    | Myo-inositol                                      | 31   |
| Dehydro-L-ascorbic acid                                | 9    | 1-kestose                                         | 32   |
| D-iso-ascorbic acid                                    | 10   | $\alpha$ -lactose. H <sub>2</sub> O               | 33   |
| Cellobiose                                             | 11   | $\beta$ -lyxose                                   | 34   |
| Methyl $\beta$ -cellobioside-methanol                  | 12   | Methyl $\beta$ -maltopyranoside. H <sub>2</sub> O | 35   |
| 2, 6-anhydro- $\beta$ -D-fructofuranose                | 13   | Isomaltulose. H <sub>2</sub> O                    | 36   |
| Galactitol                                             | 14   | D-mannitol (K Form)                               | 37   |
| D-galactono- $\gamma$ -lactone                         | 15   | $\beta$ -D-mannitol                               | 38   |
| Methyl $\alpha$ -D-galactopyranoside. H <sub>2</sub> O | 16   | Methyl $\alpha$ -D-mannopyranoside                | 39   |
| D-glucitol (A form)                                    | 17   | Planteose. 2H <sub>2</sub> O                      | 40   |
| Glucitol-pyridine                                      | 18   | Raffinose. 5H <sub>2</sub> O                      | 41   |
| D-glucono-(1, 5)-lactone                               | 19   | $\alpha$ -rhamnose. H <sub>2</sub> O              | 42   |
| 1, 6-anhydro- $\beta$ -D-glucopyranose                 | 20   | Ribitol                                           | 43   |
| $\alpha$ -D-glucose. H <sub>2</sub> O                  | 21   | $\alpha$ -L-sorbose                               | 44   |
| $\beta$ -D-glucose                                     | 22   | Sucrose                                           | 45   |
| $\alpha$ -D-glucose-urea                               | 23   | $\alpha$ , $\alpha$ -trehalose. 2H <sub>2</sub> O | 46   |
| 1, 6: 2, 3-dianhydro- $\beta$ -D-gulopyranose          | 24   | Xylitol                                           | 47   |
| Methyl $\alpha$ -D-glucopyranoside                     | 25   | $\alpha$ -xylose                                  | 48   |
| $\beta$ -D-glucurono- $\gamma$ -lactone                | 26   |                                                   |      |

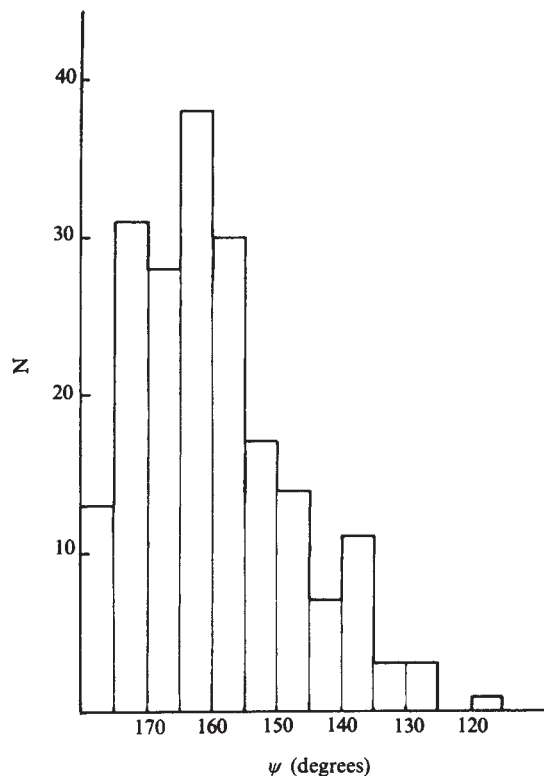


Fig. 2 Histogram of the 196 O-H...O hydrogen bond angles observed in the crystal structures listed in Table 1.

As a result of this, the maximum in the frequency distribution may be expected to occur appreciably away from 180°. In other words, conclusions about the hydrogen bond energy from angle frequency distributions can only be compared with theoretical model calculations if the spatial freedom factor is either included in the theoretical model calculations or removed from the experimental hydrogen bond angle frequency distribution.

We applied the  $\sin \psi$  correction to the statistics of a series of 196 hydrogen bond angles collected from recent literature<sup>4-48</sup>. Only one type of hydrogen bond was considered: the donor and acceptor groups were aliphatic hydroxyl groups in polyalcohols, saccharides and related compounds,

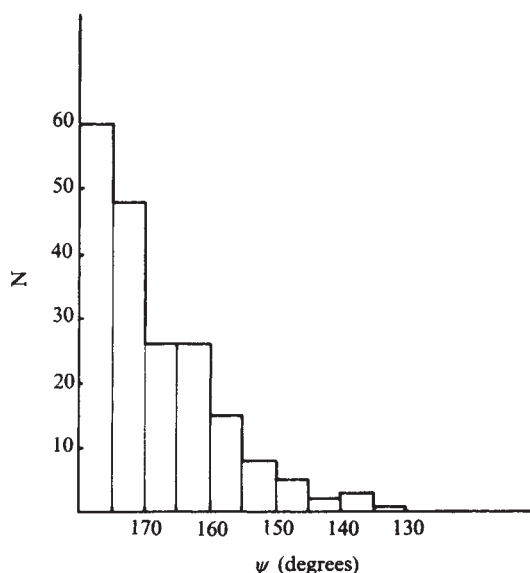


Fig. 3 The histogram from Fig. 2 after correction for the geometric  $\sin \psi$  factor.

as listed in Table 1. Whereas the histogram of  $\psi$  (Fig. 2) has a maximum at 161°, the application of the  $\sin \psi$  correction procedure produces a histogram (Fig. 3) that has a maximum at 180°.

Although distributions of experimental values of hydrogen bond angles at first sight suggest the opposite, we conclude that the experimental distributions, properly treated, are not inconsistent with an assumed preference for linear hydrogen bonds.

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Received September 26, 1973; revised February 18, 1974.

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## Effect of sliding on surface fatigue

IN hardened steels, sliding friction increases the tendency of loaded line contacts to suffer surface fatigue<sup>1</sup>, which can lead to pitting. This note describes an apparatus to study this effect in point contact and gives the first results.

The most convenient test pieces for fatigue studies are bearing balls. Our device consists of a drive ball, *a*, loaded against three radially disposed idler or intermediate balls, *i*, each of which is held in a chuck running on an angular contact ball thrust bearing. A fifth ball, *d*, is driven by these three. The contact angle between all the balls is 45° (Fig. 1).

The five-ball assembly is doubled with the driven balls mounted on opposite ends of a floating shaft and thus located without bearings. On this shaft an aluminum disk, together with two d.c. coils, acts as an eddy current brake. A double-ended motor drives both the input balls. By altering the current in the brake coils various amounts of traction or slip can be achieved at the contacts. Digital tachometers on each shaft, and strain gauges connected to suitable control circuits, automatically maintain the desired value of slip or traction. The load is axially applied by deadweight.

The contact zone between any two balls is a flat circular area. Analysis shows that for this system there is no relative motion in the contact plane between the two surfaces. This device therefore undergoes pure rolling. Sliding only occurs when traction is deliberately applied by the eddy current brake. When this was not energised, no slip could be detected

within the limits of accuracy of the measuring instruments ( $\pm 1$  part in  $10^4$ ).

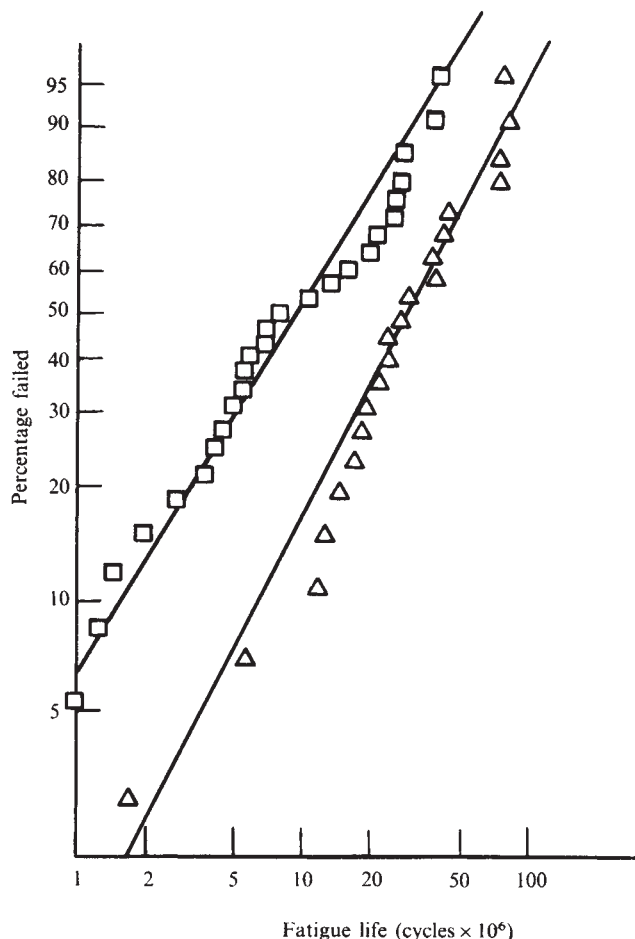


Fig. 2 Weibull plot of results.  $\Delta$ , Pure rolling;  $\square$ , 0.35% slide-roll ratio.

Using balls of EN31 (diameter  $\frac{5}{8}$  inch) and a synthetic diester lubricant of 7.5 cstokes at 96.8° C (210° F), a standard Weibull plot<sup>2</sup> was obtained at a maximum Hertz stress of 6 GN m<sup>-2</sup> (Fig. 2).

Tests were then carried out at a slide-roll ratio of 0.35% and a further plot was made. Figure 2 shows that the life decreased by 3.2 times because of this very small amount of sliding.

As no comparable apparatus exists it is not possible to see exactly how these results compare with other published data.

Tests at different contact angles<sup>3</sup>, which effectively generate different amounts of spin at the contact, have shown that on addition of small amounts of spin, the life is drastically reduced. An increase in mean spin-roll ratio from 0.168% to 0.55% reduces the life by 2.1 times. We note that the lubricant used in this case was a highly refined mineral oil quite different from the synthetic diester which we used.

The effects described here should be studied in more detail now that controlled amounts of sliding can be applied.

We thank Westland Helicopters Limited, Yeovil, for support and, especially, Squadron Leader A. Armitage of the Ministry of Defence (Procurement Executive) and Dr P. B. Macpherson for encouragement.

A full report will be published elsewhere.

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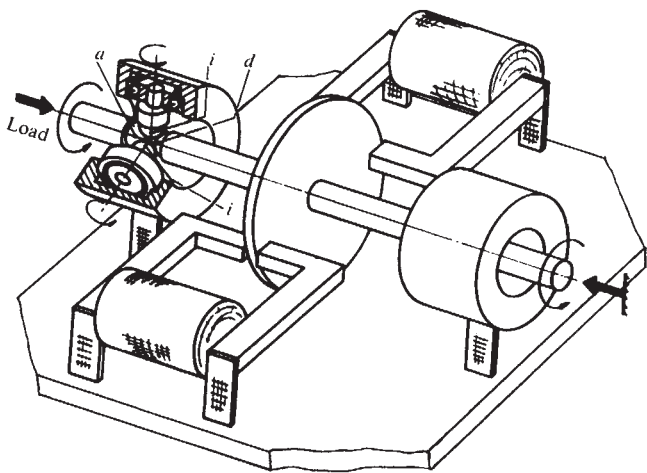


Fig. 1 Schematic twin-head five-ball assembly.

Received December 20, 1973.

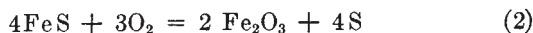
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## Is pyrophoric iron sulphide a possible source of ignition?

THERE are many practical environments in which iron oxide, in the form of surface rust, may be exposed to atmospheres containing hydrogen sulphide at various concentrations. Gas lines, chemical plant, distillation units, crude-oil cargo and storage tanks are examples.

We wish to draw attention to the hazard that can arise in such situations because of the formation of pyrophoric iron sulphide, and the possibility that it may act as a source of ignition for inflammable vapours.

The reactions involved in the formation of iron sulphide and its subsequent oxidation on exposure to atmosphere may be represented in a simplified form as follows



Both reactions are exothermic, the  $\Delta H^\circ$  values for reactions (1) and (2), based on 2 FeS, being about -40 and -151 kcalorie (-168 and -635 kJ) respectively. If reaction (2) can proceed rapidly and with little dissipation of heat, high temperatures can be expected in the material.

We have found that the reactivity of iron sulphide depends on the type of iron oxide from which it is derived. For example, a common form of rust ( $\alpha$ -FeOOH) yields on exposure to hydrogen sulphide a material which is particularly reactive. When exposed to air at ambient temperature, the material oxidizes so rapidly that surface glowing and sparking can be seen within seconds. If inflammable vapours come into contact with the material in this condition, the material can act as a source of ignition. We suspect that several recent minor explosions in the cargo tanks of crude-oil tankers may have been caused in this manner. This view is reinforced by the fact that we have been able to demonstrate that pyrophoric iron sulphide can form at partial pressures of hydrogen sulphide corresponding to the values encountered in the ullage regions of crude-oil cargo tanks.

It is fortuitous that in practical situations not all sulphidized iron oxide is as reactive as the material described. The reactivity can be influenced by a number of factors. For instance, the progressive buildup of sulphur generated during each sulphidation/oxidation cycle can exert a 'screening' effect, resulting in reduced reactivity of the sulphide so formed. Particle geometry and the presence of occluded materials can also affect reactivity.

Although pyrophoric iron sulphide has, of course, been known for many years<sup>1</sup>, the conditions under which it may form and the various factors that can influence its reactivity do not seem to have been fully explored. For this reason its potential danger may often be overlooked.

A fuller account will be given elsewhere.

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T. D. B. MORGAN  
R. W. WILSON

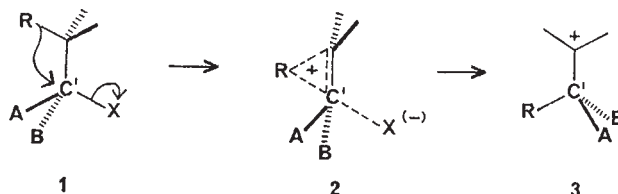
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Received February 4, 1974.

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## Stereochemistry of a carbonium ion rearrangement

CONCERTED rearrangements of the carbonium ion kind ( $1 \rightarrow 3$ ) are usually supposed to go with stereochemical inversion at the migration terminus ( $C^1$ ) and retention in the migrating group (R) (ref. 1). Many chemists have recorded one or other of these results but no one has shown both in one and the same acyclic molecule, largely because



it is very difficult to find a reaction in which both centres are chiral and both survive as chiral in the product. Rigid cyclic systems do show both results at once but this is not very informative as no other outcome is conceivable in a molecule which has no opportunity for internal rotation.

We have been studying<sup>2</sup> phosphinyl rearrangements ( $1$ ,  $R = \text{Ph}_2\text{PO}$ ), attracted by the very high yields, usually more than 95%, of single products formed in these reactions. We wanted to look at the stereochemistry at the migrating phosphorus atom, since it does not follow automatically from the carbon case that retention will be the general rule and also at carbon ( $C^1$  in  $1$ ) since we should like evidence on the detailed mechanism of the reaction: whether, for example, the two steps shown in (1) are concerted. This kind of question is worth asking because most accounts of carbonium ion rearrangements assume that electronegative groups like  $\text{Ph}_2\text{PO}$  will not migrate easily since they cannot support the positive charge in the transition state (2).

In one of our reactions<sup>2</sup> ( $4 \rightarrow 5$ ,  $R^1 = R^2 = \text{Ph}$ ), a chiral migration terminus remained chiral in the product.

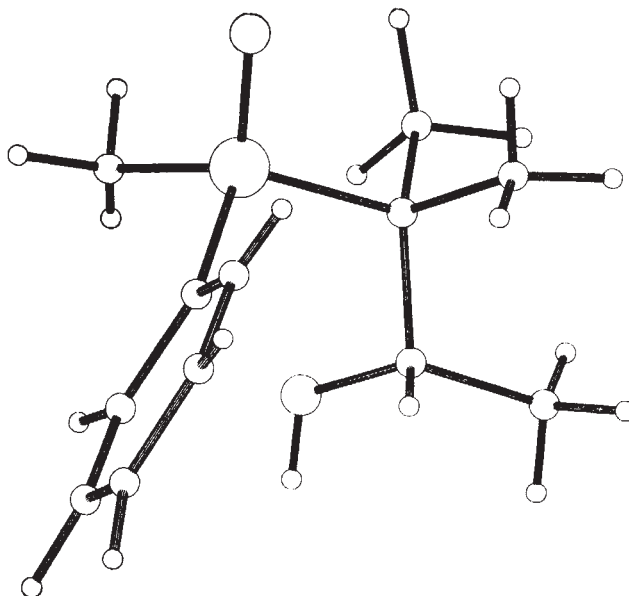


Fig. 1 Perspective drawing of the molecular structure of alcohol (7a).



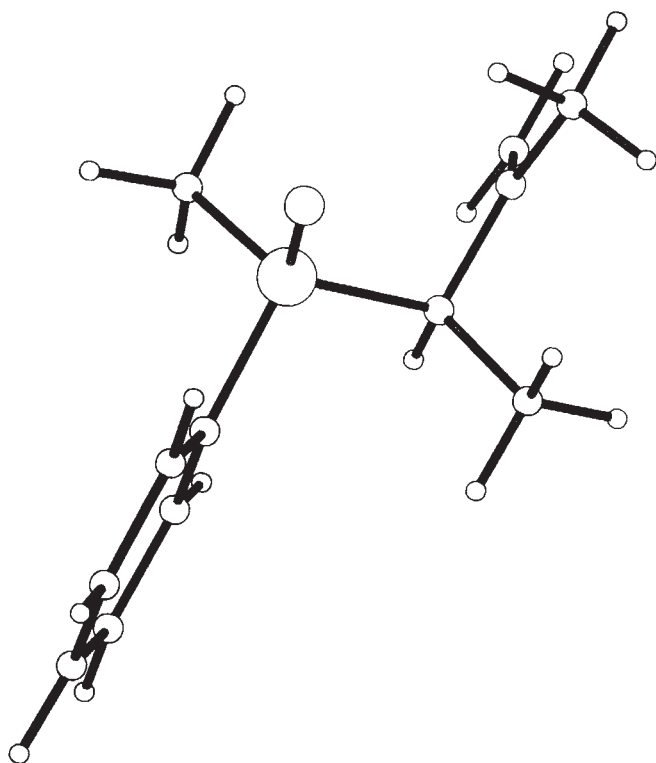
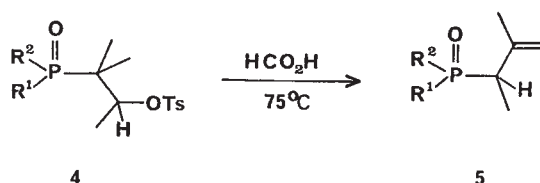
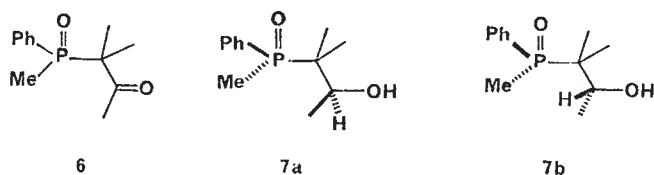


Fig. 2 Perspective drawing of the molecular structure of olefin (9a).

Simply by carrying out this reaction sequence on an analogous molecule with different substituents ( $R^1 = \text{Ph}$ ,  $R^2 = \text{Me}$ ) on phosphorus we have been able to study the stereochemistry at both chiral centres simultaneously and to do so by unambiguous X-ray crystal structure determinations since we need to distinguish diastereoisomers and not mirror images.



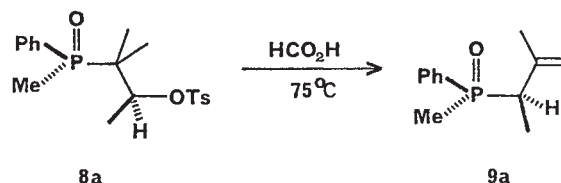
We first synthesised both diastereoisomers of the alcohol (7) by reduction of the ketone (6). One alcohol was easy to make as the bulky reducing agent  $\text{LiAlH}(\text{O}i\text{Bu})_3$  gave it in 90% yield. It formed chunky prisms from di-isopropyl ether-chloroform and its structure proved to be (7a) from the X-ray determination (Fig. 1). The other diastereoisomer (7b) was more difficult to make and in the end we separated a 60:40 mixture (from reduction with sodium borohydride



of the ketone (6)) by preparative t.l.c. of the tosylates. Tosylates (8a), made directly from alcohol (7a), and (8b) from the t.l.c. separation, had clearly distinct nmr spectra.

Tosylate (8a) solvolysed in formic acid at 75° C to give an olefin as the major product. This olefin (9a) was very soluble in water and in most organic solvents but formed

long thin trapezoidal prisms from dry 100–120° C petrol ether. Figure 2 shows the result of the X-ray determination of crystal structure. Both structure analyses were based on diffractometer data taken with monochromatised Cu radiation. The phase problem was solved by direct methods and at the current stage of refinement the reliability factors are  $R = 0.04$  for the alcohol (7a) and  $R = 0.08$  for the olefin (9a).



Some of the other diastereoisomer (9b) of the olefin is also formed in the reaction; the amount varies with time and is about 15% after ten half-lives of the tosylate. If pure olefin (9a) is dissolved in formic acid at 75° C, epimerisation to form (9b) occurs at the same rate as the formation of (9b) in the reaction mixture. So (9b) is not a product of the reaction—it is formed by epimerisation of the true product (9a). Exactly parallel results—formation of (9b) as the only product of the reaction—are found when the minor tosylate (8b) is solvolysed under the same conditions. The tosylates (8) do not epimerise under the reaction conditions.

The reaction is therefore completely stereospecific: configuration is retained in the migrating phosphorus atom and inverted at the migration terminus. This result is consistent with a concerted rearrangement: a less likely but equally valid explanation would be that tosylate leaves to give a cation and rearrangement follows more quickly than rotation of the carbon-carbon bond. Certainly the reaction must be a genuine rearrangement; there can be no question of the phosphinyl group leaving the molecule to rejoin it later at the migration terminus.

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## BIOLOGICAL SCIENCES

### Repression of RNA transcription during the development of analgesic tolerance to morphine

SEVERAL reports suggest that RNA and protein synthesis are important for the development of morphine-induced analgesic tolerance and dependency<sup>1–4</sup>. Following up these reports, we have examined the effect of chronic morphine drugging on the intracellular site of RNA synthesis-nuclear chromatin. We found that brain chromatin from rats tolerant to morphine, synthesised RNA more slowly than that from non-tolerant rats<sup>5</sup>. This effect was not caused by the direct

TABLE 1 Chromatin template activity during morphine-induced analgesia

| Treatment                          | pmol of ATP incorporated* |
|------------------------------------|---------------------------|
| Control                            | 1,136 $\pm$ 47            |
| Morphine (10 mg kg <sup>-1</sup> ) | 1,088 $\pm$ 46            |

\* Results are reported as the mean  $\pm$  s.e. for four rats. ATP incorporation by enzyme alone (340 pmol) has been subtracted.

action of morphine and was found to be associated in the chromatin complex with a protein which is believed to regulate RNA synthesis. Since we may have identified, in part, a mechanism by which morphine-induced analgesic tolerance develops, we looked for a chronological relationship between the development of morphine-induced analgesic tolerance and the effect on brain chromatin RNA synthesis.

Male Charles River rats (80–100 g) were divided into two groups: (a) morphine-treated and (b) saline control. Half the daily dose of morphine sulphate was administered subcutaneously twice daily (0800 h and 2000 h) in 0.9% NaCl. Control animals were injected at the same time with 0.9% NaCl. The initial dose of morphine sulphate was 10 mg kg<sup>-1</sup> d<sup>-1</sup>. This dose was increased progressively to 100 mg kg<sup>-1</sup> d<sup>-1</sup> on the thirteenth day so that the final doses on the first, third, sixth and thirteenth days were 10, 15, 30 and 100 mg kg<sup>-1</sup> d<sup>-1</sup>, respectively. All RNA synthesis and analgesic experiments were begun at 0800 h, 12 h after the last dose of morphine.

Analgesia induced by morphine sulphate was measured by the reaction of animals to being placed on a hot plate using a modification of the method described by Johannesson and Woods<sup>6</sup>. Rats received neither training nor predrug trails before being treated. Twelve hours after their last morphine dose, a subcutaneous 10 mg kg<sup>-1</sup> dose of morphine sulphate was given to test and control animals. After 45 min the animals were tested for analgesia. Endpoint responses were paw licking or jumping out of the cylinder with a cut-off time of 30 s. The observer was kept unaware of the treat-

ment for each group. Analgesia was registered as a delay in response to the hot plate while animals tolerant to morphine responded very rapidly.

Nuclei were isolated from individual rat brains using the methods we described previously for pooled rat brains<sup>5</sup>. Chromatin was extracted from rat brain nuclei according to the procedure of Paul and Gilmour<sup>7</sup>, and assayed for its capacity to promote RNA synthesis in the presence of *E. coli* DNA-dependent RNA polymerase according to the methods of Chamberlin and Berg<sup>8</sup>. The reaction mixture (0.5 ml) contained 10  $\mu$ M of Tris-HCl (pH 7.9), 1.0  $\mu$ M MgCl<sub>2</sub>, 3.0  $\mu$ M  $\beta$ -mercaptoethanol, 134  $\mu$ g of RNA polymerase, 10  $\mu$ g of chromatin from either control or morphine-treated animals and 0.1  $\mu$ M of UTP, CTP, GTP and ATP (0.1  $\mu$ Ci <sup>14</sup>C-8-ATP, New England Nuclear Corp.). Incubation was for 10 min at 37° C. The reaction was stopped by addition of an equal volume of cold 10% trichloroacetic acid (TCA). The precipitate was collected on Millipore filters, air dried, and counted in a toluene-PPO-POPOP cocktail using scintillation spectrometry.

To determine whether the decrease in rat brain chromatin template activity might be related to morphine-induced analgesia, brain chromatin was analysed from rats which had received 10 mg kg<sup>-1</sup> of morphine sulphate subcutaneously. At 60 min (a time when all the rats were analgesic) brain chromatin was extracted and its capacity to incorporate <sup>14</sup>C-ATP into RNA measured. Table 1 shows that brain chromatin from both control and morphine-treated rats incorporated equivalent amounts of <sup>14</sup>C-ATP into RNA even though the morphine-treated animals were analgesic.

The development of analgesic tolerance to the morphine regimen used in these experiments is shown in Fig. 1. Rats tested 1 d after morphine treatment seemed to exhibit as much analgesia to 10 mg kg<sup>-1</sup> of morphine sulphate as saline-treated rats. Analgesic tolerance became significant at 3 d and was maximal by the sixth day.

Figure 1 also shows the development of the brain chromatin depression during the same morphine regimen. After 1 d, no significant change in chromatin template activity was noted. By the third day the template activity in the morphine-treated rats was significantly depressed and this depression was maximal by the sixth day.

To examine further the relationship of analgesic tolerance and chromatin template activity, we performed hot plate and chromatin template assays at various times after the morphine regimen was stopped. Figure 2 shows the duration of analgesic tolerance produced by the 13-d morphine sulphate regimen. Seven days after the morphine regimen was discontinued analgesic tolerance was still maximal. On the twenty-eighth day, tolerance appeared to be less, but was still significantly different from control. By the thirty-fifth day, no significant analgesic tolerance was detected.

Figure 2 also shows the duration of the chromatin depression produced by the 13-d morphine regimen. Seven days after the morphine treatment was stopped, chromatin template activity was still maximally depressed. However, by the twenty-eighth day, the template activity of the morphine-treated group had increased to the level of the controls.

Our results do not support any relationship between analgesia and brain chromatin template activity, since the chromatin depression was absent during analgesia and yet maximal during analgesic tolerance.

The onset of the depressed chromatin template activity and the onset of analgesic tolerance during chronic morphine treatment seem to be related. Both became significant by the third treatment day and were maximal by the sixth day. On the other hand, when morphine treatment was stopped the depression of template activity had disappeared by the twenty-eighth day, while analgesic tolerance was still significant and did not disappear until the thirty-fifth day after

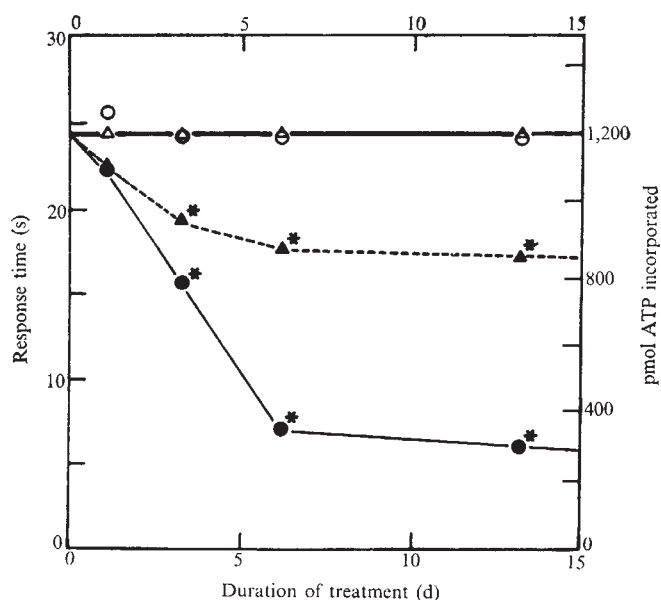


Fig. 1 Development of morphine-induced analgesic tolerance and depression of brain chromatin template activity. Each circle (○, control; ●, morphine) is the mean response time of thirteen to twenty-five rats. Each triangle (△, control; ▲, morphine) is the mean template activity of three to ten rat brains. The greatest number of rats were used at days 1, 3 and 6. \* Denotes values which are significantly different ( $P < 0.05$ ) from their respective control by Student's *t* test<sup>9</sup>.



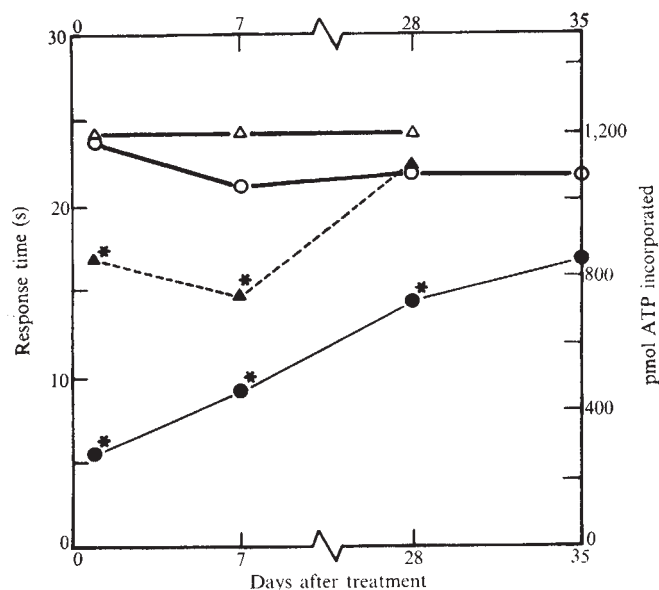


FIG. 2 Disappearance of morphine-induced analgesic tolerance and depression of brain chromatin template activity. Each circle (○, control; ●, morphine) represents the mean response time of ten to twenty-five rats. Each triangle (△, control; ▲, morphine) represents the mean template activity of three to eight rat brains. \* Denotes values which are significantly different ( $P < 0.05$ ) from their respective control by the Student's  $t$  test<sup>9</sup>.

treatment. Although chromatin template depression and analgesic tolerance did not disappear together, this does not negate a possible relationship between them. If the change in nuclear chromatin template initiates the mechanism(s) producing analgesic tolerance, then one would expect such tolerance to disappear with or after the chromatin effect.

In conclusion, we found that changes in brain chromatin template activity correlated chronologically with the onset and disappearance of morphine-induced analgesic tolerance. However, it is quite possible that the depression of chromatin template activity may be related to morphine dependency, or perhaps, it is related to both phenomena. The latter possibility seems quite possible, in light of the fundamental role of chromatin in cellular RNA synthesis and the difficulty of separating morphine-induced analgesic tolerance and dependency.

This study was supported by the US Public Health Service.

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Received November 19, 1973; revised January 17, 1974.

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## Defective rRNA synthesis in human leukaemic blast cells?

RECENT studies suggest that processing of ribosomal precursor RNA might be impaired in blast cells of acute leukaemia (AML)<sup>1,2</sup>. Following incubation with <sup>3</sup>H-methylmethionine blast cells of human acute leukaemias partially failed to methylate the 45S and 32S ribosomal precursors<sup>1</sup>. Using autoradiographic techniques, previous studies have also shown an increase in intranuclear RNA and decreased cytoplasmic RNA in these cells<sup>3</sup>. More recently, competitive hybridisation was performed with normal lymphocyte DNA and 35S-55S RNA fractions extracted from both acute leukaemia blast cells and normal PHA-stimulated lymphocytes. Using incubations with actinomycin D these experiments have led to the conclusion that leukaemic blast cells synthesise heterogeneous nuclear RNA molecules which are not transcribed in PHA-stimulated lymphocytes and it was also suggested—in contrast to the earlier data—that the amount of unmethylated unprocessed rRNA precursor was small in leukaemic blast cells<sup>4</sup>.

Studies in this laboratory have yielded highly specific <sup>32</sup>P-labelling of nuclear and ribosomal high molecular weight RNA using a HEPES-buffered, phosphate-free medium<sup>5</sup>.

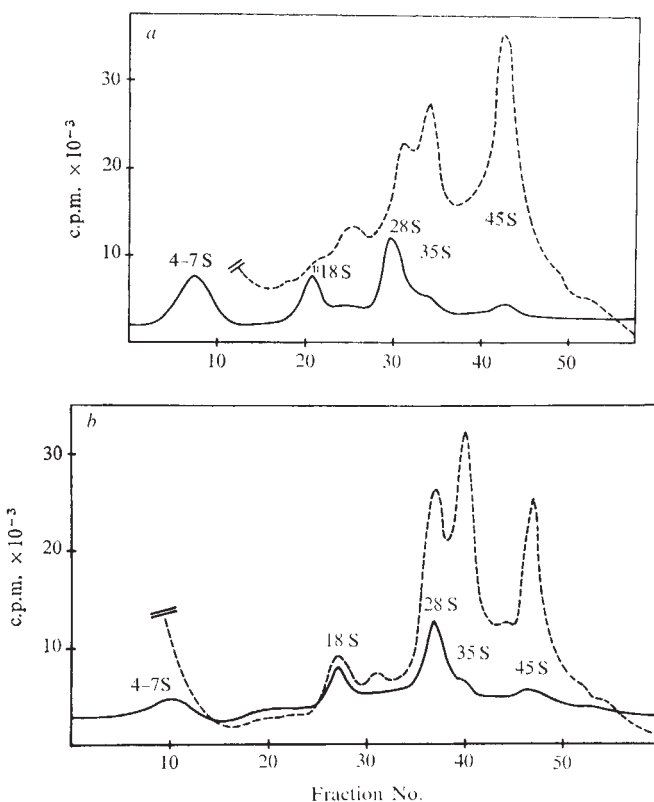


FIG. 1 *a*, Sucrose density gradient centrifugation of nuclear RNA from acute myeloblastic leukaemia cells. The cells were labelled for 6 h in a phosphate-free medium<sup>5</sup>. Nuclei were isolated by homogenisation in 5% citric acid and purified by sucrose step gradients<sup>6</sup>. The RNA was extracted by the hot phenol-SDS-method<sup>7</sup> at 65° C for 5 min. RNA (1 mg) was loaded on a 6%-45% (w/w) sucrose density gradient (Beckman Density Gradient Former) and centrifuged for 16 h at 26,000 r.p.m. (3° C, SW27 rotor). The RNA was fractionated with an ISCO Model 640 Density Gradient Fractionator and aliquots of 20  $\mu$ l were counted in Bray's solution in a Packard Model 3380 Tricarb Liquid Scintillation Spectrometer to determine the distribution of radioactivity. *b*, Sucrose density gradient centrifugation of nuclear RNA from PHA-stimulated normal lymphocytes. Lymphocytes were derived from normal donors by separation on nylon fibre columns<sup>10</sup>. PHA stimulation was done according to the method of Cooper<sup>10</sup>. For details of RNA extraction and separation see (*a*).

TABLE 1 Comparative specific activities of  $^{32}\text{P}$ -labelled nuclear 45S RNA and 28S rRNA from PHA-stimulated lymphocytes and human leukaemic blast cells

| Tissue                            | c.p.m. per mg RNA |                   | Specific activity    |
|-----------------------------------|-------------------|-------------------|----------------------|
|                                   | Nuclear 45S RNA   | 28S rRNA          | 45S nRNA<br>28S rRNA |
| AML (Patient N., H.)              | $3.2 \times 10^8$ | $4.7 \times 10^6$ | 68.1                 |
| AML (Patient N., K.)              | $2.8 \times 10^8$ | $2.4 \times 10^6$ | 116.2                |
| AML (Patient L., J.)              | $1.5 \times 10^9$ | $9.3 \times 10^6$ | 161.0                |
| PHA-stimulated normal lymphocytes |                   |                   |                      |
| Experiment 1                      | $1.5 \times 10^9$ | $1.6 \times 10^8$ | 9.4                  |
| 2                                 | $1.2 \times 10^9$ | $1.7 \times 10^8$ | 7.1                  |
| 3                                 | $1.3 \times 10^9$ | $1.4 \times 10^8$ | 9.3                  |

The conditions were standardised in that each gram of packed leukaemic cells derived from heparinised blood of untreated leukaemia patients was incubated at 37° C with 50 mCi  $^{32}\text{P}$ -orthophosphate using a 1:100 ratio (w/w) of cells and medium. Nuclei were isolated after 6 h using 5% citric acid to prevent RNase degradation as described by Dounce<sup>6</sup>. The purified nuclear pellet was subjected to controlled hydrolysis with 1.0 mg highly purified DNase (Boehringer) per g nuclei in 1.5 ml 0.5 M NaCl, 0.05 M  $\text{MgCl}_2$ , 0.001 M Tris, pH 7.4, at 22° C for 3 min. Buffer (40 ml) containing 0.1 M NaCl, 0.01 M Na acetate, 0.001 M  $\text{MgCl}_2$ , pH 5.1, was added to 1 g of packed nuclei and the suspension was made 0.34% in sodium dodecylsulphate. The mixture was shaken with hot phenol at 65° C for 5 min as described by Scherrer and Darnell<sup>7</sup>. After two re-extractions with hot phenol, undegraded, highly labelled nuclear RNA was precipitated with two volumes of ethanol as demonstrated by sucrose density gradient centrifugations (Fig. 1a and b). A marked label was found in the 45S region and, in all experiments, only the three peak fractions were collected.

In a different batch, 1 g of cells derived from the same patient was incubated for 9 h using identical conditions and cytoplasmic RNA was extracted from a ribosomal preparation as described previously<sup>8</sup>. To eliminate the possibility of degradation, rRNA preparations were only used when they met the basic requirements of purity and quality as outlined by Attardi and Amaldi<sup>9</sup>.

Lymphocytes stimulated with PHA were prepared as described by Cooper<sup>10</sup>. In all experiments with both leukaemia cells and PHA-stimulated lymphocytes, nuclear 45S RNA and 28S rRNA were characterised by the determination of specific activities, nucleotide composition analyses, and oligonucleotide frequency studies after complete digestion of the RNA fractions with pancreatic RNase. The techniques of column chromatography on DEAE-Sephadex A25 and high voltage paper electrophoresis have been described elsewhere<sup>11,12</sup>. The details of comparative structural studies of high molecular weight nuclear and rRNA species from various human leukaemias and lymphomas will be reported elsewhere (S.S., K.P.B., J.K., C.G.S. and H. Busch, unpublished).

Figure 1 shows the distribution of radioactivity in nuclear RNA fractions when separated on 6%–45% sucrose density gradients (pH 5.1) and fractionated into 58–60 portions of 0.6 ml. Some differences were found between the proportion of label in 45S, 35S and 28S RNA with a higher percentage of label in 45S in the case of AML (Fig. 1a and b). High specific activities up to  $1.5 \times 10^9$  c.p.m. per mg RNA were

determined for the 45S fraction both from PHA-stimulated normal lymphocytes and leukaemic blast cells (Table 1). No major differences were found in nucleotide composition analyses (all run in triplicate) between two of three cell lines of human leukaemia and the transformed normal lymphocytes (Table 2). Table 2 also indicates that the RNA found in the 45S fraction was mainly of the preribosomal type with a G+C/A+U ratio of 1.96–2.00. Table 1 summarises the specific activities of nuclear 45S RNA and ribosomal 28S RNA from three individual experiments each with acute myeloblastic leukaemia cells and PHA-stimulated normal lymphocytes.

The striking differences found in the ratios of the specific activities, as well as the demonstration of very small amounts of label in 28S rRNA of blast cells clearly indicate a failure of rRNA synthesis in the leukaemia cells. Although there were some consistent differences in the  $^{32}\text{P}$ -nucleotide compositions with equal labelling of guanylic and cytidylic acid in the case of the blast cells (the values for a number of tissues reported by Attardi and Amaldi<sup>9</sup> correspond to those found for PHA-stimulated lymphocytes in our studies) no significant structural differences were found between 28S rRNA of leukaemic and non-leukaemic cells when the frequencies were determined for the partial sequences A-Cp, A-Up, G-Cp, G-Up, A-A-Cp, (A, G)-Cp, A-A-Up, G-G-Cp, G-A-Up, A-G-Up and G-G-Up (S.S., K.P.B., J.K., C.G.S., and H. Busch, unpublished).

These results, based on high specific labelling of nuclear and cytoplasmic RNA species, support the previous findings of a block in processing of the ribosomal precursor to the RNA of the mature ribosome in blast cells of human acute leukaemia. This condition, however, may not be unique to leukaemic blast cells since it has also been pointed out that processing of the ribosomal precursor may be different in actively growing cells, resting cells and cells under transition from the resting state to active growth<sup>13</sup>. It may be of interest that with the use of the same techniques we found similar small amounts of label in cytoplasmic RNA in acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL). Conversely, high specific activities of 28S rRNA were determined in two cases of leukaemic lymphosarcoma and in a Burkitt lymphoma cell line (unpublished).

The transformation of chronic lymphocytic leukaemia into lymphosarcoma which has been reported many times may be, on a cellular level, characterised by an increased processing of preribosomal RNA. With our preparations of RNA from a monosome-polysome pellet we never found disproportionate labelling of 18S and 28S RNA with rela-

TABLE 2  $^{32}\text{P}$ -Nucleotide compositions of nuclear 45S RNA and 28S rRNA from PHA-lymphoblasts and leukaemic blast cells

| Tissue                     | 28S rRNA |      |      |      | s.e. | 45S nRNA |      |      |      | s.e. |
|----------------------------|----------|------|------|------|------|----------|------|------|------|------|
|                            | A        | U    | G    | C    |      | A        | U    | G    | C    |      |
| AML (Patient N., H.)       | 15.7     | 16.2 | 34.6 | 33.6 | <0.6 | 14.5     | 19.1 | 32.8 | 33.6 | <0.5 |
| AML (Patient N., K.)       | 15.7     | 17.4 | 33.2 | 33.7 | <0.4 | 13.8     | 18.1 | 34.2 | 33.9 | <0.4 |
| AML (Patient L., J.)       | 17.1     | 15.4 | 33.9 | 33.6 | <0.3 | 18.1     | 21.5 | 29.8 | 30.7 | <0.6 |
| PHA-stimulated lymphocytes | 16.4     | 15.9 | 35.8 | 31.9 | <0.3 | 14.5     | 20.1 | 32.5 | 32.8 | <0.5 |



tively small amounts of label present in the 18S rRNA. It has been postulated that the assembly of ribosomes in normal and also leukaemic lymphocytes would be controlled by such a 'wastage' of 18S rRNA<sup>14,15</sup>. These studies were, however, based on RNA extractions from unfractionated lymphocytes and showed very low label in the 18S fraction of resting normal and leukaemic lymphocytes.

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## Presence of a large poly(rC) tract within the RNA of encephalomyocarditis virus

THE replication of the RNA-containing bacteriophages has been found to involve specific replicases, partly coded by the phage genome (reviewed in refs 1 and 2). The replication proceeds by way of a partially double-stranded intermediate form, and the replicases must presumably recognise the 3'-terminal regions of the RNA (+) and (-) strands as start points for copying<sup>1,2</sup>. There is also evidence that the Q $\beta$  replicase recognises a specific internal region of the Q $\beta$  RNA molecule, which is involved in forming a replicative complex<sup>3</sup>. Replication of the smallest animal RNA viruses, the picornaviruses, also requires specific replicases, and has a mechanism<sup>4-7</sup> which is not dissimilar to that of the RNA phages, as far as it has been elucidated. We have been studying nucleotide sequences of regions of a picornavirus RNA which might be specifically recognised by its replicase, or have specific roles in the replicative process, in the same way that such sequences have been characterised in the RNA phages<sup>1-3</sup>.

Encephalomyocarditis (EMC) virus, which is typical of the cardiovirus group of picornaviruses, contains a continuous single-stranded RNA molecule of molecular weight around  $2.7 \times 10^6$ , about 7600 nucleotides in length (refs 8 and 9 and D. Frisby, unpublished). We have found a region of 95-100 nucleotides in the interior of the molecule, con-

taining 85-90 C residues. Here we describe the isolation and characterisation of this poly(rC) region, and discuss its possible significance.

EMC virus was cultivated in Krebs ascites cells in the presence of <sup>32</sup>P-orthophosphate, and radioactive virus was purified from the cell lysate in the way described in the legend to Fig. 1. The RNA was extracted from the purified virus by phenol-chloroform treatment. Subsequent sucrose gradient centrifugation of the RNA revealed a single peak, shown in Fig. 1, with a sedimentation coefficient of about 37S, corresponding to the size previously reported for EMC RNA (refs 8 and 9 and D. Frisby, unpublished). Electrophoresis of this RNA peak through 4% polyacrylamide gels containing formamide yielded a single high molecular weight band, demonstrating the absence of any hidden breaks in the purified material. We therefore suggest that the products

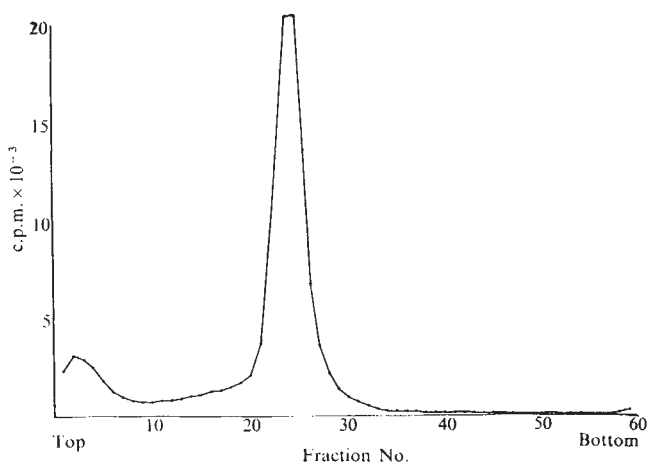


Fig. 1 Sucrose gradient profile of <sup>32</sup>P-labelled EMC RNA. The RNA was prepared as follows. A 100 ml suspension of washed Krebs ascites cells in HB-NE medium (New Earle's medium, containing in g l<sup>-1</sup>: NaCl, 6.28; KCl 0.37; MgSO<sub>4</sub> 7 H<sub>2</sub>O, 0.18; phenol red, 0.0009; glucose, 1.8; CaCl<sub>2</sub> 6 H<sub>2</sub>O, 0.18; NaHCO<sub>3</sub>, 3.3; penicillin G, 0.06; and streptomycin, 0.06; buffered to pH 7.5 with HEPES) containing  $1.5 \times 10^7$  cells ml<sup>-1</sup> was infected with EMC virus at a multiplicity of 10 PFU per cell. To enable viral multiplication, the infected cell suspension was allowed to stand at 22° C for 0.5 h, then gently shaken in a 1 l flask for 3 h at 37° C. <sup>32</sup>P-orthophosphate was then added to a concentration of 0.1 mCi ml<sup>-1</sup>, and shaken for a further 16-18 h during viral multiplication. The resulting lysate was centrifuged at 16,000g for 0.5 h at 4° C (MSE centrifuge) to remove cell debris. Labelled virus was pelleted from the supernatant by centrifugation (110,000g) for 2 h at 4° C (in the Beckman L2-50 ultracentrifuge) and the pellet partially resuspended in 1 to 2 ml of PBS-A buffer (containing in g l<sup>-1</sup>: NaCl, 8.0; KCl, 0.2; Na<sub>2</sub>HPO<sub>4</sub>, 1.15 g; KH<sub>2</sub>PO<sub>4</sub>, 0.2, pH 7.3), then left overnight at 4° C. Several volumes of PP8 buffer (0.1 M NaCl, 0.1 M K<sub>2</sub>HPO<sub>4</sub> and 0.2 M Na<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, 10 H<sub>2</sub>O, pH 8.0) were added to this suspension to solubilise the virus, and the total volume was made up to 25 ml with additional PBS-A. The suspension was then layered onto a 10 ml sucrose cushion (29% sucrose in PBS-A) and centrifuged at 110,000g as above, but for 3 h. The second virus pellet was resuspended in PBS-A together with an equal volume of PP8, as described above, and layered onto an 11 ml 10.2-22.4 % isokinetic sucrose gradient in PBS-A buffer, and centrifuged for 75 min at 36,000 r.p.m. at 4° C in the SW41 rotor. Over 95% of the radioactivity on the gradient was always found in the 160S virus peak, which was extracted with equal volumes of Tris buffer-saturated phenol (pH 7.5) and chloroform, in the presence of 0.5% sodium dodecyl sulphate. The <sup>32</sup>P-labelled EMC RNA was precipitated from the aqueous phase by the addition of 0.5 mg of unlabelled carrier RNA, 0.1 volume of 2 M sodium acetate and 3 volumes of ethanol. This RNA was sedimented through a further similar sucrose gradient for 3.5 h, the profile of which is shown above. About 50 µg of labelled RNA with a specific activity of  $0.5-0.8 \times 10^6$  c.p.m. µg<sup>-1</sup> was usually obtained.

TABLE 1 Analysis of the large  $T_1$  RNase resistant oligonucleotides from EMC RNA. The products arising upon complete pancreatic RNase digestion of fragments  $A_0$ - $A_3$  and  $\alpha$  (Fig. 3) were excised, and the radioactivities of the spots on paper were determined directly by scintillation counting, enabling the quantities of the products, relative to Gp, to be estimated (column 3). All the estimates are calculated from the mean values of three determinations, and enable the lengths of the oligonucleotides to be approximately estimated, given in column 4. These lengths are consistent with the mobilities of the fragments relative to each other, and to the electrophoretic front in 10% polyacrylamide, and suggest that the fragments are homogeneous. This is also supported by the amounts of the fragments released upon hydrolysis of the RNA, about one mole of each per mole of RNA (Column 5). Their yields were determined by counting a small aliquot of an RNA digest (0.8%) before gel electrophoresis, and then counting the fractionated fragments following their elution from polyacrylamide onto DEAE-paper discs (this part of the elution procedure is quantitative since in multiple experiments the amount of residual  $^{32}\text{P}$  counts remaining in the gel slice compared with that eluted onto the DEAE-paper discs was always less than 0.5%. No leakage of  $^{32}\text{P}$  into the anode buffer compartment was detectable). Taking the length of the RNA as 7600 nucleotides (refs 8 and 9 and D. Frisby, unpublished), the total radioactivity of the RNA digest applied to the gel may be calculated in terms of c.p.m. per nucleotide, and thus also the expected radioactivities of fragments of these sizes, if each is present once. The observed molarities may then be calculated from comparison of the observed radioactivities with the expected radioactivities (Columns 5 and 6). In a typical experiment,  $8.6 \times 10^6$  c.p.m. of digested EMC RNA were applied to a gel slab. The radioactivities of the fragments eluted were determined to be 92,000 c.p.m. ( $A_0$ ), 36,700 c.p.m. ( $A_1$ ), 46,300 c.p.m. ( $A_2$ ) and 39,400 c.p.m. ( $A_3$ ).

| Fragment | Analysis of fragment |             | Chain length and yield of fragment |                                              |                                                  |
|----------|----------------------|-------------|------------------------------------|----------------------------------------------|--------------------------------------------------|
|          | Oligonucleotide      | Yield found | Suggested length (Nucleotides)     | Abundance in EMC (moles per moles RNA) Found | Abundance in RNA (moles per moles RNA) Suggested |
| $A_0$    | AAC                  | 0.7         | 95-100                             | 0.9                                          | 1                                                |
|          | AC                   | 0.9         |                                    |                                              |                                                  |
|          | G                    | 1.0         |                                    |                                              |                                                  |
|          | C                    | 87          |                                    |                                              |                                                  |
|          | U                    | 4.1         |                                    |                                              |                                                  |
| $A_1$    | AAAAC                | 0.8         | 36.41                              | 1.0                                          | 1                                                |
|          | AAU                  | 1.5         |                                    |                                              |                                                  |
|          | AAC                  | 1.5         |                                    |                                              |                                                  |
|          | AU                   | 2.6         |                                    |                                              |                                                  |
|          | AC                   | 1.2         |                                    |                                              |                                                  |
|          | G                    | 1.0         |                                    |                                              |                                                  |
|          | C                    | 5.9         |                                    |                                              |                                                  |
|          | U                    | 4.5         |                                    |                                              |                                                  |
| $A_2$    | AAU                  | 1.5         | 26-31                              | 1.4                                          | 1                                                |
|          | AAC                  | 1.5         |                                    |                                              |                                                  |
|          | AC                   | 1.0         |                                    |                                              |                                                  |
|          | G                    | 1.0         |                                    |                                              |                                                  |
|          | C                    | 7.9         |                                    |                                              |                                                  |
|          | U                    | 6.2         |                                    |                                              |                                                  |
| $A_3$    | AAAAC                | 1.0         | 27-31                              | 1.2                                          | 1                                                |
|          | AAU                  | 0.8         |                                    |                                              |                                                  |
|          | AAC                  | 1.0         |                                    |                                              |                                                  |
|          | G                    | 1.0         |                                    |                                              |                                                  |
|          | C                    | 7.1         |                                    |                                              |                                                  |
|          | U                    | 8.5         |                                    |                                              |                                                  |
| $\alpha$ | ?                    | —           | }                                  | 0.3                                          |                                                  |
|          | ?                    | —           |                                    |                                              |                                                  |
|          | ?                    | —           |                                    |                                              |                                                  |
|          | AAG(?)               | 0.1         |                                    |                                              |                                                  |
|          | AAU                  | 0.2         |                                    |                                              |                                                  |
|          | AAC                  | 0.3         |                                    |                                              |                                                  |
|          | AG                   | 0.3         |                                    |                                              |                                                  |
|          | AU                   | 0.5         |                                    |                                              |                                                  |
|          | AC                   | 0.7         |                                    |                                              |                                                  |
|          | G                    | 1.0         |                                    |                                              |                                                  |
|          | C                    | 12.4        |                                    |                                              |                                                  |
|          | U                    | 2.0         |                                    |                                              |                                                  |

arising from subsequent nuclease digestion of this RNA cannot be derived from any contaminating low molecular weight material within the virus particles, whether or not associated with the EMC RNA.  $^{32}\text{P}$ -labelled EMC RNA was completely digested with  $T_1$  RNase, and the resulting products were fractionated through a large slab of 10% polyacrylamide gel containing 7 M urea, as described in the legend to Fig. 2. The products were located by autoradiography (Fig. 2).

The majority of the oligonucleotides migrate close behind the electrophoretic front, and are in the size range 1-25 nucleotides, as expected. Three larger products,  $A_1$ ,  $A_2$  and  $A_3$  are also found, of approximately 25-40 nucleotides. This also corresponds to the expected abundance of oligonucleotides of this size arising upon  $T_1$  RNase digestion of an RNA of the size and composition of EMC. A further product,  $A_0$ , however, containing 95-100 nucleotides by

analysis, is also present. This is much larger than any oligonucleotide which would be expected to arise from an RNA of 7,600 nucleotides in a random sequence, since the probability of a section of this length lacking any G residues is vanishingly small. Finally a further band,  $\alpha$ , apparently slightly larger than  $A_0$ , is found in small and variable amounts among the digestion products.

We have analysed the fragments  $A_0$ - $A_3$  and  $\alpha$  by further digestion with pancreatic RNase. The results of these analyses are reported in Table 1, and the fractionation of the products is shown in Fig. 3. The gel fractionation indicates that fragment  $A_2$  must be a few nucleotides larger than  $A_3$ , but our analyses are not sufficiently precise to show the exact size difference. Fragments  $A_1$ ,  $A_2$  and  $A_3$  display no unusual structural features, as judged from the results of these partial analyses. In particular, there is no preponderance of any one nucleotide in them.



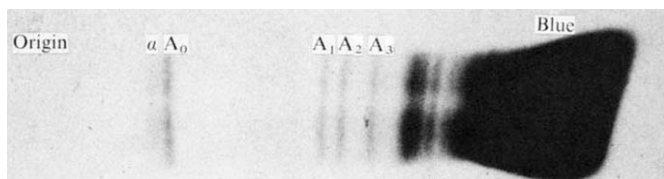


FIG. 2 Fractionation of the products arising upon complete hydrolysis of EMC RNA with  $T_1$  RNase.  $2-40 \times 10^6$  c.p.m. of  $^{32}\text{P}$ -labelled EMC RNA (5–50  $\mu\text{g}$ , containing 500  $\mu\text{g}$  unlabelled carrier tRNA) was dissolved in 0.02 ml of 0.02 M Tris HCl pH 7.4; 0.002 M EDTA (low salt buffer) containing  $T_1$  RNase (Sankyo Ltd., Japan) at an enzyme: substrate (w/w) ratio of 1:40. Digestion was continued for a further 1 h at  $37^\circ\text{C}$ . Alternatively digestion was carried out in 0.02 M Tris HCl-0.02 M  $\text{MgCl}_2$  (high salt buffer) at pH 7.5. No difference in the digestion pattern was observed. The RNA digest was mixed with an equal volume of 7 M urea containing 60% sucrose and a trace of bromophenol blue, and layered into a  $0.1 \times 1 \times 5$  cm pocket at the top of a 10% polyacrylamide gel slab (measuring  $0.3 \times 15 \times 40$  cm) made up in the following buffer: 0.1 M Tris borate, pH 8.3; 0.0025 M EDTA and 7 M urea. Electrophoresis was carried out in the cold for 16–18 h at 500 V and 30 mA. The radioactive bands were located by autoradiography, as shown above, and excised using the autoradiograph as a guide. Fragments  $A_0$ – $A_3$  and  $\alpha$  were eluted electrophoretically onto DEAE-cellulose paper discs which were washed in ethanol and counted to estimate the molar yields of the fragments (see legend to Table 1). Elution of the labelled fragments from the discs was performed using freshly-prepared 30% triethylammonium carbonate, pH 10, as described by Adams *et al.*<sup>25</sup>

In contrast, the very large product  $A_0$  contains 85–90 C residues, together with 3 As, 4 Us and the expected 3' terminal Gp. The estimated size of 95–100 nucleotides derived from this analysis is consistent with its position on the 7 M urea gel relative to the other fragments  $A_1$ – $A_3$ . Estimates of the sizes of these are also included in Table 1. We have also determined the yields of the fragments  $\alpha$  and  $A_0$ – $A_3$  in terms of the numbers of moles of each which arise from each mole of EMC RNA upon enzymatic hydrolysis. These values are reported in Table 1. It may be seen that all of the fragments except  $\alpha$  are equimolar, and each seems to occur once in the molecule.

The analysis of band  $\alpha$  is also listed in Table 1. From its position on gel electrophoresis, the material contained in it is a few nucleotides longer than the fragment  $A_0$ . On the basis of a length of 100 nucleotides it is present in roughly 0.3 M amounts in digests of the RNA. It has a complex composition. Like  $A_0$ , it is very rich in C, but this may arise by contamination with  $A_0$  because of 'tailing' of the latter fragment. It also contains more than one product terminating in Gp, in addition to Gp itself, which suggests that it might be a mixture of two or more components, each present in very small amounts in the digest. These might arise because of incomplete hydrolysis of a small proportion of the RNA, and this view is supported by the variable amounts of  $\alpha$  present in digests. Perhaps this is due to incomplete deproteinisation of the RNA affording specific nuclease-resistant areas. This is purely speculative, however.

So far we have no direct evidence about the location of the poly(rC) tract within the EMC RNA; however an indirect argument suggests that it might be situated close to one of the termini. The aggregate molecular weight of the EMC virus-specified proteins has been estimated to be about  $2.3 \times 10^5$  (ref. 10). The molecular weight of the RNA necessary to code for these proteins would be about  $2.3 \times 10^6$ . Since the molecular weight of the EMC RNA is around  $2.7 \times 10^6$ , there are roughly 1,000 nucleotides of untranslated RNA within the EMC viral genome. These

untranslated regions must be situated at the termini of the RNA, since the initial translation product is a single polypeptide<sup>10</sup>; that is, there are no untranslated areas between the cistrons. The poly(rC) tract would code for a polypoline region if it were translated. The amount of proline present in the proteins of the EMC viral capsid is relatively small<sup>11</sup>; however, we cannot exclude the possibility that a polypoline tract is present in some other virally-specified protein within the host cell cytoplasm, although this seems unlikely. This suggests that the poly(rC) region is probably untranslated, and so occurs fairly close to one or other of the termini of the RNA molecule. If so, it would account for roughly 10% of the untranslated nucleotides.

Poly(rC)-enriched regions do not exist in bacterial tRNAs<sup>12</sup> or rRNAs<sup>13,14</sup>, nor have they been detected within small phages such as Q $\beta$  or R17 (refs 1 and 2 and A. G. Porter, unpublished). In the case of Q $\beta$  RNA (A. G. Porter, unpublished), oligonucleotides resulting from complete  $T_1$  RNase digestion were fractionated by electrophoresis followed by homochromatography<sup>15,16</sup>. None of the largest  $T_1$  RNase fragments (15–25 nucleotides) were found to be unusually rich in any one nucleotide. Preliminary analyses of the longest oligonucleotides arising from  $T_1$  RNase digestion of influenza viral RNA and tobacco mosaic viral RNA<sup>17,18</sup> have also not revealed poly(rC). Since poly(rC) areas have not been detected in any other naturally occurring RNAs, can any specific function be attributed to this region within the EMC RNA? Two observations suggest that the poly(rC) might be part of a site recognised by the EMC viral replicase at some stage in the replicative process. First, it has recently been reported<sup>19</sup> that a partially purified EMC viral replicase preparation will synthesise poly(rG) from a poly(rC) template. Second, the Q $\beta$  replicase, which has subunits of very similar size to those of the EMC replicase<sup>19</sup>, can also synthesise poly(rG) using a poly(rC) template, but cannot use other homopolymers or natural nucleic acids as templates, except the Q $\beta$  RNA itself<sup>20,21</sup>. As mentioned above, however, the Q $\beta$  RNA does not contain a  $T_1$  RNase-resistant tract of poly(rC) comparable in length with that of EMC RNA (refs 1 and 2 and A. G. Porter, unpublished), and therefore the significance of this observation remains unclear. The suggestion that the poly(rC) region in EMC RNA might function in replication is reinforced if, as seems likely, the poly(rC) tract lies close to one of the termini in a non-coding area of the RNA.

About 60% of the nucleotides in the EMC RNA participate in secondary structure when the RNA is in free solution (D. Frisby and R. Cotter, unpublished). If the poly(rC) tract is involved in this secondary structure, a corresponding area rich in poly(rG) would be expected in the EMC RNA. Complete digestion of the RNA with pancreatic RNase, however, does not give products longer than 15–20 nucleotides (our unpublished results), excluding the presence of long uninterrupted tracts of poly(rG). Therefore any secondary structure involving the poly(rC) region would contain substantial mismatching, although no conclusions can yet be drawn regarding the presence or absence of such secondary structure.

No products containing extended tracts of poly(A) occur among the large oligonucleotides arising from  $T_1$  RNase digestion of EMC RNA in either high salt or low salt conditions. These ought to be detectable in the same way as other large oligonucleotides, if present, and it therefore seems that the RNA, at least within the virus particle, lacks any appreciable poly(A). Similar findings have been reported for the closely related cardiovirus Mengo<sup>22</sup>, where the maximum length of the poly(A) tracts has been estimated to be about 16 nucleotides. We have not yet systematically examined the smaller EMC RNA oligonucleotides in this size range, and therefore we do not know whether any

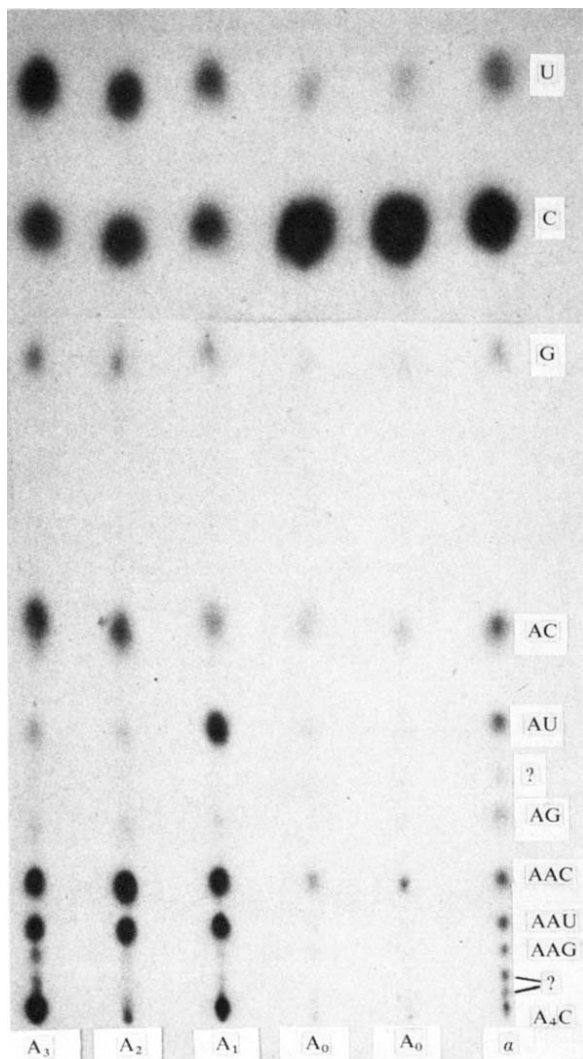


Fig. 3 Fractionation of the products of pancreatic RNase digestion of the large fragments from EMC RNA. The fragments  $A_0$ - $A_3$  and  $\alpha$  (Table 1) were completely digested with pancreatic RNase as described by Brownlee *et al.*<sup>15,16</sup> and the products fractionated by electrophoresis on DEAE paper at pH 3.5 (0.5% pyridine-5% acetic acid). The positions of the radioactive spots were determined by autoradiography, as shown above, and the oligonucleotides quantified by counting and eluted<sup>15,16</sup>. In most cases, the pancreatic oligonucleotides could be identified from their positions on the autoradiograph. In all cases, however, firm identification was made (Table 1) by total alkaline hydrolysis of every oligonucleotide, followed by fractionation of the products by electrophoresis on Whatman No. 52 paper at pH 3.5 (refs 15 and 16). In one experiment, oligonucleotides arising from pancreatic RNase digestion of fragments  $A_0$ - $A_3$  were separated by two-dimensional electrophoresis (on cellulose acetate at pH 3.5 and DEAE paper in 7% formic acid<sup>15,16</sup>). The pancreatic RNase digest of fragment  $A_0$  was fractionated in two tracts in order to obtain a mean count of each oligonucleotide present.

short poly(A) stretches are present in the EMC RNA. The coronaviruses therefore seem to differ in this respect from polio, an enterovirus, which has been reported to contain about 50 (ref. 23) or 90 (ref. 24) A residues at its 3'-terminus.

We are now examining the RNAs of a number of other picornaviruses for the presence of poly(rC) tracts, and attempting to determine the location of the poly(rC) region in the EMC RNA directly by sequencing.

We thank C. Newton for technical assistance, D. Frisby, I. Lindley, Dr N. Stebbing and Dr A. E. Smith for their valuable discussions and help. We also thank Drs H. Gould

and M. Szekely for very kindly providing electrophoresis equipment while we awaited delivery of our own.

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Received November 27, 1973; revised January 17, 1974.

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## In vivo and in vitro effect of $\alpha$ -amanitin on preimplantation mouse embryo RNA polymerase

THE effects of various metabolic inhibitors, including actinomycin D, puromycin, cycloheximide, mitomycin C, and fluorophenylalanine, on preimplantation mouse embryos have been previously studied<sup>1-6</sup>, to investigate when RNA synthesis begins in preimplantation embryos and what types of RNA are synthesised during the various stages of development. Monesi and Salfi<sup>3</sup> originally reported that there was no appreciable overall RNA synthesis until the morula stage. Ellem and Gwatkin<sup>7</sup> also found no detectable RNA synthesis at the two-cell stage of development. Recently, using more



TABLE 1 Effect of  $\alpha$ -amanitin on development of two-cell mouse embryos 48 h after commencing  $\alpha$ -amanitin treatment.

| Dose $\alpha$ -amanitin<br>( $\mu\text{g ml}^{-1}$ ) | Total No.<br>Embryos | No. 2-cell<br>Embryos | No. 3-4 cell<br>Embryos | No. morula-<br>blastocysts | % arrested<br>at 2-cell |
|------------------------------------------------------|----------------------|-----------------------|-------------------------|----------------------------|-------------------------|
| 0.00                                                 | 96                   | 16                    | 16                      | 64                         | 17                      |
| 0.03                                                 | 47                   | 18                    | 19                      | 10                         | 38                      |
| 0.3                                                  | 33                   | 15                    | 16                      | 2                          | 45                      |
| 1.0                                                  | 304                  | 220                   | 82                      | 2                          | 71                      |

sensitive techniques, RNA synthesis has been detected as early as the two-cell stage of development<sup>8-10</sup>. We assumed that since RNA synthesis occurs in preimplantation mouse embryos, the enzyme, DNA-dependent RNA polymerase, must be active. We report here the results of *in vivo* and *in vitro* experiments intended to characterise more fully the RNA polymerase present in preimplantation mouse embryos. We used the mushroom toxin,  $\alpha$ -amanitin, to determine which forms of RNA polymerase are active and the relative amounts of each form which are present in preimplantation mouse embryos.

In an earlier study<sup>11</sup> we reported the existence of three forms of DNA-dependent RNA polymerase in the nuclei of adult mouse liver. It was determined that two of the forms, IA and IB, were resistant to an  $\alpha$ -amanitin concentration of  $0.3 \mu\text{g ml}^{-1}$  whereas form II was completely inhibited by this amount of toxin. These findings are in general agreement with those of Roeder and Rutter<sup>12</sup> in which it was further determined that form I polymerase is found primarily in the nucleolus and synthesises ribosomal-like RNA while form II is found in the nucleoplasm and synthesises a DNA-like RNA. We thought it should be possible, then, to ascertain whether RNA polymerase form II is active in synthesising mRNA in preimplantation mouse embryos.

A first set of experiments was designed to test the *in vivo* effects of  $\alpha$ -amanitin. Preimplantation mouse embryos at the two-cell stage of development were collected from CF1 female mice (Carworth Labs, Inc., Portage, Michigan) after superovulation with 5 IU pregnant mare serum (PMS), (Organon) followed 48 h later by 5 IU human chorionic gonadotropin (HCG), (Nutritional Biochemical). The embryos were collected and washed in Whitten and Biggers medium<sup>13</sup> and cultured in microdrops of medium (containing one or two embryos) under oil which had been preequilibrated with the medium. The cultures were maintained at  $37^\circ\text{C}$  in an atmosphere of 5%  $\text{CO}_2$ , 5%  $\text{O}_2$ , 90%  $\text{N}_2$ . When  $\alpha$ -amanitin was used as an inhibitor, it was added to the medium in which the embryos were collected, and was present throughout all further manipulation including equilibration of the oil. The embryos were scanned visually for stage of development 48 h after being introduced into microdrop culture. The results of these experiments are shown in Table 1. It is seen that 17% of the normal embryos failed to develop past the two-cell stage under our culture conditions. The effect of  $\alpha$ -amanitin was to arrest development at the two-cell stage with  $1 \mu\text{g ml}^{-1}$  of  $\alpha$ -amanitin arresting 71% of the embryos

tested. It is interesting to note that although 67% of the untreated embryos developed to the morula or blastocyst stages less than 1% of the embryos which had been treated with  $1 \mu\text{g ml}^{-1}$  of  $\alpha$ -amanitin developed to the morula or blastocyst stages.

From the *in vivo* experiments reported above it seems that RNA polymerase is indeed active as early as the two-cell stage of development in the mouse. Exactly which of the multiple RNA polymerases is inhibited remains an open question. Although it is generally agreed that *in vitro* only the nucleoplasmic form of the enzyme is inhibited, Tata *et al.*<sup>14</sup> have shown that *in vivo* both the nucleoplasmic and nucleolar polymerases may be inhibited.

For *in vitro* experiments with solubilised enzyme, CF1 mice were superovulated as described earlier and blastocysts collected 86-92 h after HCG injection. The embryos were treated with Pronase to remove the zona pellucida, washed three times in Whitten and Biggers medium, washed once in  $0.01 \text{ M}$  Tris pH 7.4, 0.5% BSA, and taken up in  $10 \mu\text{l}$  of the same buffer. The embryos were frozen and thawed three times in liquid nitrogen.  $2.5 \mu\text{l}$  of  $2.75 \text{ M}$   $(\text{NH}_4)_2\text{SO}_4$  (pH 7.9) were then added to bring the  $(\text{NH}_4)_2\text{SO}_4$  concentration to  $0.55 \text{ M}$ .  $25 \mu\text{l}$  of TGMED ( $0.05 \text{ M}$  Tris-HCl, pH 7.9; 25% glycerol;  $5 \text{ mM}$   $\text{MgCl}_2$ ;  $0.1 \text{ mM}$  EDTA;  $0.5 \text{ mM}$  dithiothreitol) or TGMED containing the desired concentration of  $\alpha$ -amanitin were then added followed by  $37.5 \mu\text{l}$  of assay mixture. The final reaction mixture ( $75 \mu\text{l}$ ) contained:  $36 \mu\text{g}$  calf thymus DNA,  $50 \text{ mM}$  Tris-HCl, pH 7.9,  $2 \text{ mM}$   $\text{MnCl}_2$ ,  $1.6 \text{ mM}$   $\text{MgCl}_2$ ,  $6 \text{ mM}$  NaF,  $8 \text{ mM}$  KCl,  $0.5 \text{ mg ml}^{-1}$  BSA,  $0.3 \text{ mM}$  spermine,  $0.5 \text{ mM}$  dithiothreitol,  $0.6 \text{ mM}$  ATP,  $0.6 \text{ mM}$  GTP,  $0.6 \text{ mM}$  CTP,  $0.004 \text{ mM}$   $^3\text{H}$ -UTP (New England Nuclear, specific activity  $26.9 \text{ Ci mmol}^{-1}$ ),  $3 \mu\text{g}$  pyruvate kinase (Sigma,  $320 \text{ units mg}^{-1}$ ),  $4 \text{ mM}$  phosphoenolpyruvate,  $0.09 \text{ M}$   $(\text{NH}_4)_2\text{SO}_4$ , 8.3% glycerol,  $0.03 \text{ mM}$  EDTA. The reaction mixture was incubated at  $37^\circ\text{C}$  for 40 min and the amount of incorporated  $^3\text{H}$ -UMP determined by the method of Litman<sup>15</sup>.

The RNA polymerase activity of blastocysts in the presence and absence of  $1.1 \mu\text{g ml}^{-1}$   $\alpha$ -amanitin is shown in Table 2. We show data from two experiments, B12 and B13. The average values reported as  $^3\text{H}$ -UMP incorporated per embryo per min ( $\text{pmol} \times 10^{-5}$ ) are  $2.70 \pm 0.42$  without  $\alpha$ -amanitin and  $1.83 \pm 0.29$  with  $\alpha$ -amanitin. This corresponds to 32% inhibition of enzyme activity by  $1.1 \mu\text{g ml}^{-1}$   $\alpha$ -amanitin. The average values for experiment B12 alone are 3.09 without  $\alpha$ -amanitin and 2.01 with  $\alpha$ -amanitin, whereas the average values for experiment B13 alone are 2.44 without  $\alpha$ -amanitin and 1.57 with  $\alpha$ -amanitin. This corresponds to 35% inhibition in experiment B12 and 36% inhibition in experiment B13. We have found that these types of experiments are always internally consistent, but absolute values may vary from one experiment to another, as illustrated by the data for experiments B12 and B13. For this reason our experiments always include several samples without  $\alpha$ -amanitin as a control.

Figure 1 shows the inhibition of mouse blastocyst RNA polymerases by varying doses of  $\alpha$ -amanitin. The assays were all performed at high ionic strength, which favours the detection of form II RNA polymerase. When these experiments were repeated at low ionic strength, the results shown in Table 3 were obtained. It is seen that the blastocyst enzymes are inhibited 6% at low ionic strength compared

TABLE 2 Effect of  $\alpha$ -amanitin on RNA polymerase activity from mouse blastocysts

| Experiment No. | No. embryos | $\alpha$ -amanitin<br>( $1.1 \mu\text{g ml}^{-1}$ ) | ( $\text{pmol} \times 10^{-5}$ )<br>$^3\text{H}$ -UMP incorporated<br>per embryo per min |
|----------------|-------------|-----------------------------------------------------|------------------------------------------------------------------------------------------|
| B12            | 100         | —                                                   | 2.96                                                                                     |
| B12            | 100         | —                                                   | 3.22                                                                                     |
| B12            | 100         | +                                                   | 1.97                                                                                     |
| B12            | 100         | +                                                   | 1.80                                                                                     |
| B12            | 100         | +                                                   | 2.26                                                                                     |
| B13            | 100         | —                                                   | 2.22                                                                                     |
| B13            | 100         | —                                                   | 2.75                                                                                     |
| B13            | 134         | —                                                   | 2.34                                                                                     |
| B13            | 100         | +                                                   | 1.57                                                                                     |
| B13            | 100         | +                                                   | 1.57                                                                                     |

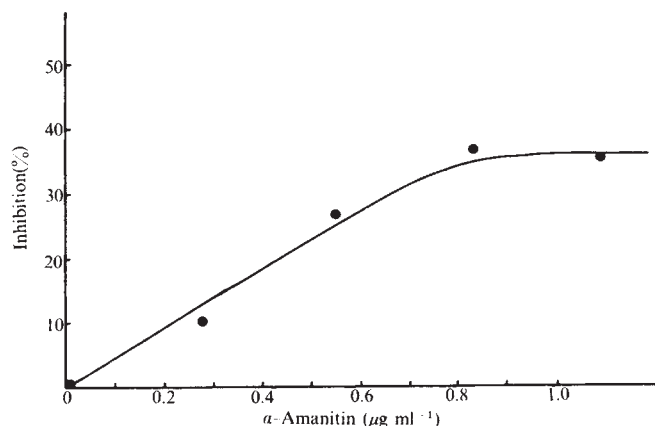


FIG. 1 Inhibition of RNA polymerase activity against concentration of  $\alpha$ -amanitin for mouse blastocyst enzymes.

with a maximum of 35% at high ionic strength. In contrast, the adult fraction 4 (F4) enzymes showed 50% maximal inhibition at low ionic strength and 82% maximal inhibition at high ionic strength. We also found that a concentration of 0.30 mg ml<sup>-1</sup> causes maximal inhibition of F4 and form II adult enzymes, while a concentration of 0.8  $\mu$ g ml<sup>-1</sup> of  $\alpha$ -amanitin was necessary for maximal inhibition of the embryonic enzymes (compare Fig. 1 with Table 3). Therefore, it seems that the blastocyst enzymes are less sensitive to inhibition by  $\alpha$ -amanitin than are the adult liver enzymes.

There are several possible explanations for these observations. First, it is possible that differences in the solubilisation process for the liver and embryonic polymerases may alter their properties. A second possibility which may effect the  $\alpha$ -amanitin sensitivity of the enzymes is the amount of endogenous template present before the addition of  $\alpha$ -amanitin. In the embryo experiments no attempt was made to remove the endogenous template while there is very little or no endogenous template present in the solubilised liver enzyme preparation (unpublished observation).

The most attractive possibility is that the difference in maximal inhibition of total RNA polymerase solubilised from blastocysts (35%) and liver nuclei (82%) may indicate that the relative proportions of form I and form II polymerases are different in adult and embryonic tissues and that more form I is present (relative to form II) in blastocysts than in adult liver nuclei. This, as well as the *in vivo* results, represents presumptive evidence for the direct involvement of the various forms of RNA polymerase in the regulation of RNA synthesis in preimplantation mouse embryos.

The question of whether the number of RNA polymerase molecules per cell correlates with the amount of RNA syn-

thesised at any given stage in embryonic development is controversial. Siracusa<sup>16</sup> has reported in a preliminary note that there is no such correlation in mouse embryos from the fertilised egg to the blastocyst stage. Roeder *et al.*<sup>17</sup> also found no such correlation in *Xenopus laevis*, but did find a correlation between the rate of RNA synthesis and the levels of RNA polymerase per cell in the sea urchin<sup>18</sup>.

The control of RNA synthesis, however, may be more dependent on the types of RNA polymerase present in early embryos, than on the total amount of enzyme present. The former could specifically control which RNA species were synthesised at any given time, whereas the latter would at best be a reflection of total RNA synthesis. We have shown that at least two distinct forms of RNA polymerase activity, based on their inhibition by  $\alpha$ -amanitin, are present in preimplantation mouse embryos. We are currently trying to show which types of RNA are synthesised by the different forms of the enzyme at various stages in preimplantation mouse development.

We thank Professor T. Wieland for the  $\alpha$ -amanitin. This work was supported by a grant from the Population Council, New York.

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Received November 5; revised December 27, 1973.

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TABLE 3 Comparison of effect of  $\alpha$ -amanitin on embryonic and adult enzymes at high and low ionic strength.

| Enzyme*<br>preparation | $\alpha$ -amanitin<br>( $\mu$ g ml <sup>-1</sup> ) | (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub><br>(M) | % inhibition<br>of control |
|------------------------|----------------------------------------------------|--------------------------------------------------------|----------------------------|
| F4                     | 0.30                                               | 0.01                                                   | 50                         |
| F4                     | 0.30                                               | 0.04                                                   | 73                         |
| F4                     | 0.30                                               | 0.09                                                   | 82                         |
| IA                     | 0.30                                               | 0.04                                                   | 0                          |
| IB                     | 0.30                                               | 0.05                                                   | 0                          |
| II                     | 0.01                                               | 0.10                                                   | 72                         |
| II                     | 0.30                                               | 0.10                                                   | 100                        |
| blastocyst             | 1.1                                                | 0.03                                                   | 6                          |
| blastocyst             | 1.1                                                | 0.09                                                   | 35                         |

\* F4, is the mixture of RNA polymerases from adult mouse liver directly before DEAE-Sephadex chromatography; Forms IA, IB and II are the isolated enzyme forms after DEAE-Sephadex chromatography (see Versteegh and Warner<sup>11</sup>). Blastocyst enzyme is as described in the text.

## Bacteriophage of *Halobacterium salinarum*

*Halobacterium salinarum* is a member of the Halobacteria, a group of obligate, extremely halophilic organisms requiring at least 15% NaCl in their growth media. No bacteriophage has previously been found in association with members of this group.

*H. salinarum* strain 1 was cultivated as described by Dundas *et al.*<sup>1</sup>. During an investigation of flagella from *H. salinarum*, phage particles were observed in some crudely purified flagellar preparations. Phage-containing preparations did not give rise to plaques when plated with exponentially growing *H. salinarum* cells. Attempts at ultraviolet induction of *H. salinarum* cultures resulted in normal death curves



without any concomitant phage production. Maintenance of batch cultures, started from isolated *H. salinarum* colonies, in the logarithmic phase of growth, by means of serial transfer of cultures in late log phase to fresh media, resulted in eventual lysis of the cultures. Lysis may occur after the first transfer or be delayed for more than six transfers.

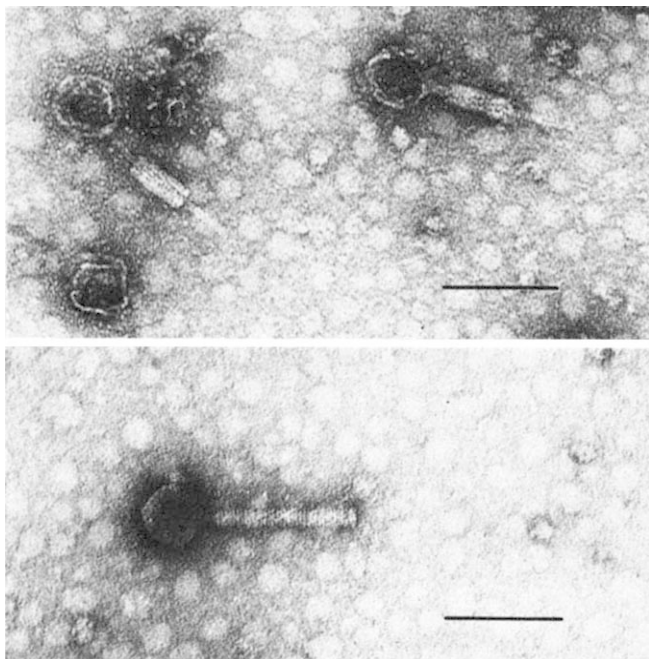


Fig. 1 Electron micrographs of preparations from *H. salinarum* culture lysates. All preparations were negatively stained with uracil acetate. The scale bar is 0.1  $\mu$ m.

In these experiments the cultures initially exhibited normal generation times of 6.1 to 6.7 h. Before lysis, culture generation time increased to more than 10 h. At this point cultures would lyse in 24-48 h after entering late logarithmic growth phase. Lysis took place both in cultures stored without aeration at room temperature and in cultures incubated with aeration at 37°C. Normal cultures (generation time 6-7 h) will not lyse when incubated for more than a week with or without aeration, at 37°C or at room temperature.

All culture lysates produced in this manner contained phage particles. (Fig. 1). Phage particles have not yet been found by routine electron microscopic examination of normal cultures at any stage of growth. The phage particles have an isometric polyhedral head and a contractile tail. The dimensions of the particles are quite uniform.

The existence of bacteriophage for *Halobacterium* opens the existing possibility of further exploration of the genetic basis of extreme halophilism.

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Received November 23; revised January 21, 1974.

## Further observations on the structure of the lipid-containing bacteriophage PM2

RECENT work on the proteins of bacteriophage PM2 have led us to a model for the structure of this virus, proposed in this communication. Experimental results have enabled us to assign each of the four proteins<sup>1</sup> to morphological structures of the virus revealed by electron microscopic and structural studies. Packing considerations indicate that protein II must form the outer protein shell<sup>2</sup>. Both proteins I and II are on the outside of the phage particle as demonstrated by labelling with radioactive iodine after lactoperoxidase treatment<sup>3</sup> by the method of Phillips and Morrison<sup>4,5</sup>.

In agreement with these results we have now shown that protein I can be selectively removed by digestion with bromelain, a proteolytic enzyme. Furthermore protein II can be labelled with <sup>35</sup>S-sulphanilic acid-diazonium salt by the method of Berg<sup>6</sup> (Fig. 1). We have also isolated a nucleocapsid core by dissociation of the virion in 4.5 M urea. This core, which has an approximate  $S_{20,w} = 133$  and which has been identified by electron microscopy as a well-organised icosahedrally-shaped core structure, contains all protein III and IV, as well as the viral DNA, but no phospholipids and

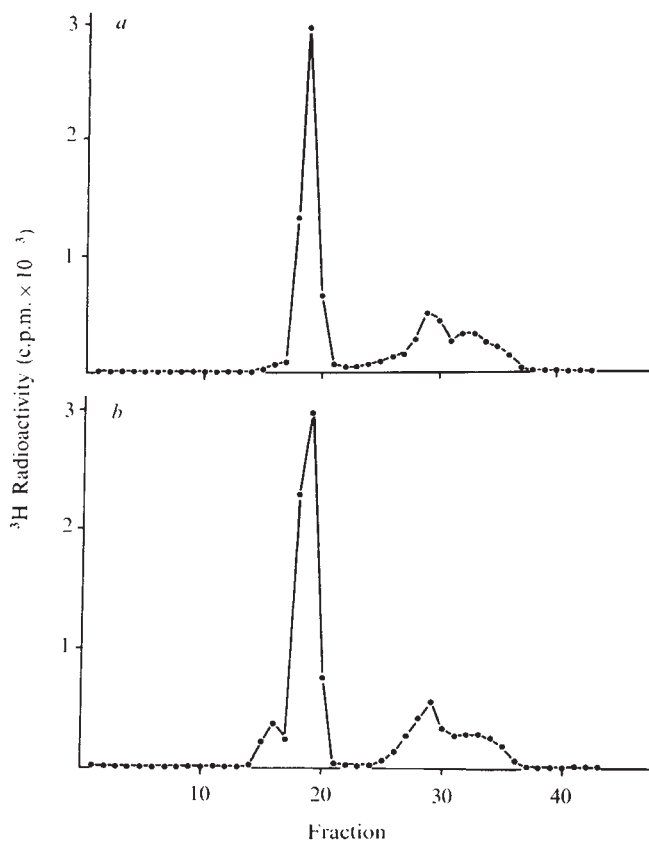


Fig. 1 a, Digestion of protein I by bromelain <sup>3</sup>H-amino acid-labelled PM2 virus was added to 1 ml of 0.01 M sodium phosphate buffer (pH 7.0), containing 0.1 M NaCl, 0.001 M CaCl<sub>2</sub>. After the addition of 1 mg bromelain, the mixture was incubated at 30° C for 3 h. The ratio of PM2 protein to protease was 0.1. At the end of the incubation, the mixture was chilled on ice. The protease was separated from PM2 on a sucrose step gradient, containing 20%, 40%, and 60% sucrose layers. Centrifugation was carried out at 120,000g for 1 h. After centrifugation, the virus band was collected and subjected to SDS-gel electrophoresis<sup>1</sup>. In a control experiment (b) the virus was prepared for gel electrophoresis by the same procedures as described above, without digestion with the proteolytic enzyme. In both cases, after gel electrophoresis the gels were frozen and sliced into 0.9 mm fractions. The slices were placed in glass vials containing 8 ml toluene-PPO-POPOP-mixture and 3% Protosol. Vials were incubated with shaking overnight at 37° C and counted in a Packard liquid scintillation counter.

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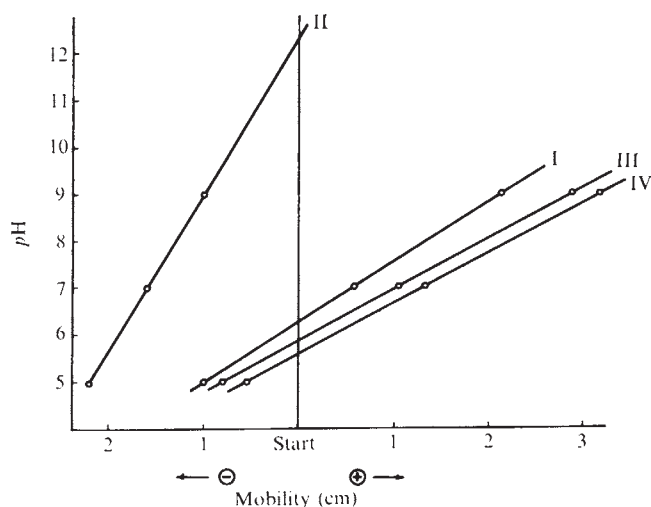


Fig. 2 Estimation of the isoelectric points of the individual PM2 proteins by cellulose-acetate electrophoresis. Before electrophoresis, cellulose-acetate sheets ( $4 \times 17$  cm) were equilibrated for 10 min in separation buffers (0.5 M Tris acetate pH 5, 7, or 9; 6 M urea, 0.02 M 2-mercaptoethanol). Protein solutions (5–20  $\mu$ l) (10–20 mg ml<sup>-1</sup> PM2 virus) were applied to the gel with a polyethylene capillary tip. Electrophoresis at the different pH values were carried out in a moist chamber on a refrigerated Teflon-coated surface for 90 min at constant voltage (40 V cm<sup>-1</sup>). After electrophoresis, the protein was stained with a 0.25% (w/v) amido black 10B solution which contained methanol, water, acetic acid (45:45:10 (v/v)). The solvent (without amido black) was used for destaining. The running distances of the individual PM2 polypeptides and reference proteins (not shown) were plotted against the different pH values. To determine the isoelectric points these mobilities were extrapolated to zero mobility.

no protein I and II. Thus protein II, the major viral protein, forms the outer icosahedral shell; proteins III, IV and the DNA the inner icosahedral shell of the nucleocapsid, and the phospholipid bilayer is sandwiched between these two shells. Protein III probably forms the protein bridges<sup>7</sup> which connect the inner and outer shells through the bilayer. From preliminary electron microscopic studies and from experiments showing that the phage cannot bind to its host cell after digestion of protein I, we imagine that protein I must form the outer spikes found at the vertices of the icosahedron.

We also measured the isoelectric points of the four viral proteins. These were determined on disrupted virus particles by electrophoresis on cellulose acetate strips at various pH values. The mobilities were extrapolated to zero mobility, a method preferable to isoelectric focusing in which the virus proteins precipitate near the isoelectric point. Whereas proteins I, III, and IV have isoelectric points at slightly less than neutrality (pH 6.2, 5.8, and 5.5, respectively), the isoelectric point of protein II is 12.3 (Fig. 2). Thus whereas proteins I, III, and IV are slightly negatively charged at pH 7.0, protein II is strongly positively charged.

From previous studies, PM2 contains 12.6–14.0% of phospholipid, of which 64% is phosphatidylglycerol (PG) and 27% is phosphatidylethanolamine (PE)<sup>8,9</sup>. These ratios of phospholipids are essentially the inverse of those of the host cell (23% PG and 75% PE)<sup>8</sup>. As PG is negatively charged and PE is neutral at neutral pH values, we propose that electrostatic interactions between protein II and PG must play a major role in the structural integrity as well as in the assembly of phage PM2. We predict that there must be a very strong asymmetry in the bilayer, with PG preferentially located in the outer lamella of the bilayer. From calculations of the number of PG molecules and the amount of positive charge due to protein II there is more than enough positive charge to neutralise the charge on PG, as well as

the excess negative charge of proteins I, III, and IV. Experiments to test the hypothesis of bilayer asymmetry, as well as experiments to determine the electrophoretic mobility of the virion are now in progress. Bilayer asymmetry has already been demonstrated for the erythrocyte membrane<sup>10,11</sup>.

From a study of the origin of the viral phospholipids it is clear that the difference in viral phospholipid composition from that of the host cell must be due to selective processes occurring during virus assembly<sup>12</sup>. There are active selective processes (biochemical processes) involving phospholipid synthesis and hydrolysis<sup>12</sup>. There must also be passive (physical) selective processes and we propose that the electrostatic interaction of protein II with PG must play a major part in this latter process. Our present studies on PM2 structure, synthesis, and assembly should provide valuable information on protein-protein and protein-lipid interactions in the formation of one type of natural bilayer.

This work was supported by a grant from the Swiss National Fund.

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Received November 20, 1973; revised January 14, 1974.

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## Fucosylglycolipids in cells transformed by a temperature-sensitive mutant of murine sarcoma virus

WE have reported that fucosylglycolipid metabolism is markedly altered in cells transformed by oncornavirus<sup>1</sup>. Transformed cells exhibit a sharp decrease in radioactive fucose incorporation into a chromatographically less mobile fucosylglycolipid, with a corresponding increase in radioactivity in chromatographically more mobile fucosylglycolipids. We describe here further studies of fucosylglycolipid metabolism using normal rat kidney (NRK) cells transformed by a cold-sensitive mutant of murine sarcoma virus (MSV)<sup>2</sup>. The cell line, designated NRK (MSV-1b), is cold sensitive for maintenance of the transformed phenotype. The cells express the transformed phenotype at the permissive temperature (39°C) but appear phenotypically normal at the non-permissive temperature (33°C). Cells transformed by this mutant are non-producer cells, but contain the MSV genome in a rescueable form. Using these cold-sensitive transformants, we were able to demonstrate, in a temporal fashion, a relationship between altered fucosylglycolipid metabolism and the development of the transformed phenotype.



TABLE 1 Incorporation of  $^{14}\text{C}$ -fucose into glycolipids of normal (NRK), NRK (MSV-1b) at non-permissive and permissive temperatures, and transformed NRK (MSV-MLV) cell lines

|                               | 1<br>NRK<br>(normal) |                          | 2<br>NRK (MSV-1b) at 33°C<br>(non-permissive) |                          | 3<br>NRK (MSV-1b) at 39°C<br>(permissive) |                          | 4<br>NRK (MSV-MLV)<br>(transformed) |                          |
|-------------------------------|----------------------|--------------------------|-----------------------------------------------|--------------------------|-------------------------------------------|--------------------------|-------------------------------------|--------------------------|
|                               | %<br>Compound        | c.p.m. per<br>mg protein | %<br>Compound                                 | c.p.m. per<br>mg protein | %<br>Compound                             | c.p.m. per<br>mg protein | %<br>Compound                       | c.p.m. per<br>mg protein |
| Fucosylglyco-<br>sphingolipid |                      |                          |                                               |                          |                                           |                          |                                     |                          |
| FucCer I                      | 1.6                  | 121                      | 1.3                                           | 100                      | 1.5                                       | 91                       | 3.8                                 | 316                      |
| FucCer II                     | 2.3                  | 174                      | 4.0                                           | 308                      | 4.6                                       | 280                      | 5.6                                 | 466                      |
| FucCer III                    | 24.0                 | 1814                     | 27.6                                          | 2127                     | 65.8                                      | 3999                     | 80.5                                | 6700                     |
| FucCer IV                     | 72.0                 | 5443                     | 67.0                                          | 5163                     | 28.1                                      | 1702                     | 10.1                                | 832                      |

Culture conditions and thin-layer chromatography are described in Fig. 1. Compounds were located by autoradiography on Kodak No-screen X-ray films, scraped and quantitated by scintillation spectroscopy. Protein determination was carried out by the Lowry method. Permissive temperature (that is, phenotypically transformed) was 39°C; non-permissive temperature (that is, phenotypically normal) was 33°C.

All cells were grown in glass bottles in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum (v/v). Cell monolayers were washed three times with phosphate-buffered saline (PBS), removed by scraping into PBS and sedimented to a pellet. The pellet was washed with PBS and the lipid was extracted<sup>1</sup>. The fucosylglycolipids were characterised as previously described<sup>1</sup>, with the additional observation that pronase treatment<sup>3</sup> had no effect on the chromatographic behavior of the fucosylglycolipids.

To investigate fucosylglycolipid metabolism at permissive and non-permissive temperatures, cells were seeded in medium supplemented with 0.5  $\mu\text{Ci ml}^{-1}$  of U- $^{14}\text{C}$ -L-fucose (133 mCi mmol<sup>-1</sup>, (Amersham/Searle, Arlington Heights, Illinois) and were grown at either 33°C or 39°C until they reached confluence ( $\sim 72$  h). On a protein basis, comparable amounts of radioactivity were incorporated into the fucosylglycolipids of uninfected NRK cells, the NRK (MSV-1b) cells grown at the permissive or non-permissive temperature, and into NRK cells transformed by the Moloney leukaemia-sarcoma virus complex (NRK (MSV-MLV), Table 1). Approximately equal amounts of radioactivity were chroma-

tographed (Fig. 1), and the major radioactive areas were excised from the plate and counted (Table 1). Consistent with the previous study<sup>1</sup> of other oncornavirus-transformed cell lines, there was a considerable alteration in the pattern of fucose labelling of glycolipids of the virus-producing, transformed NRK (MSV-MLV) cells (Fig. 1, lane 4; Table 1, column 4) compared with uninfected NRK cells (Fig. 1, lane 1; Table 1, column 1). NRK (MSV-MLV) cells exhibited a marked decrease in the amount of the chromatographically less mobile fucosylglycolipid (FucCer IV) with a concomitant increase in the chromatographically more mobile FucCer III. The fucosylglycolipid patterns of uninfected NRK cells and of NRK (MSV-MLV) cells were the same in cells cultured at 33°C or 39°C. Examination of the fucosylglycolipid pattern of cold-sensitive NRK (MSV-1b) cells grown at permissive temperature (Fig. 1, lane 3; Table 1, column 3) revealed a pattern which resembled the transformed NRK (MSV-MLV) cells. However, the inhibition of synthesis of the chromatographically less mobile component (FucCer IV) was not as marked in the NRK (MSV-1b) cells grown at permissive temperature as in the NRK (MSV-MLV) cells. In this regard, other measurements at permissive temperature (for example, sugar transport and saturation density)<sup>4</sup> suggest less expression of viral transforming function in cells transformed by the cold-sensitive mutant compared with cells transformed by the parental strain of MSV-MLV. Examination of the fucosylglycolipid pattern of NRK (MSV-1b) cells grown at the non-permissive temperature (Fig. 1, lane 2; Table 1, column 2) demonstrated a striking resemblance to the pattern observed with normal cells.

We next measured the effect of temperature shift of NRK (MSV-1b) cells on fucosylglycolipid synthesis. Based on the results of the above experiments, reciprocal temperature-shift studies were performed to examine, in a temporal fashion, the relationship between fucosylglycolipid metabolism and the appearance of the transformed phenotype. Twenty-four hours after shifting to the permissive temperature, there were no marked morphological changes, and the fucosylglycolipid profile was comparable with cultures maintained at nonpermissive temperature (Fig. 2a and b, 24 h). After 48 h most cells were morphologically transformed and the fucosylglycolipid pattern of these cultures resembled that of cultures maintained continuously at the permissive temperature rather than of those maintained continuously at the non-permissive temperature (Fig. 2, a compared with b and c, 48 h). There was an increase in FucCer III, and decreases in FucCer I, II and IV. After 72 h the cultures shifted to the permissive temperature were essentially all transformed and the fucosylglycolipid pattern was the same as that of cultures maintained continuously at the permissive temperature (Fig. 2b and c, 72 h). On the other hand, 24 h after the reciprocal shift from permissive to non-permissive temperature, both the morphology and fucosylglycolipid pattern (Fig. 2d, 24

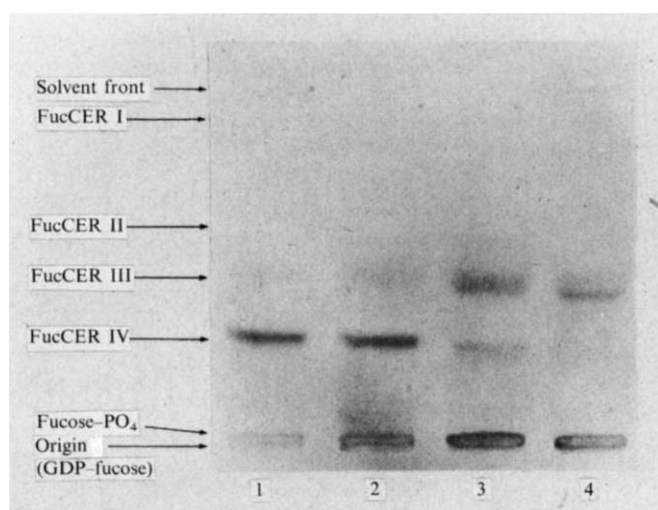


Fig. 1 Autoradiogram of the  $^{14}\text{C}$ -fucose-labelled glycolipids from: (1) NRK cells, (2) NRK (MSV-1b) cells grown at 33°C (non-permissive temperature), (3) NRK (MSV-1b) cells grown at 39°C (permissive temperature) and (4) NRK (MSV-MLV) transformed cells. Culture conditions and lipid extraction are described in the text. Lipids were chromatographed on hard silica gel plates 'Q5' (Quantum Industries, New Jersey) in 2-propanol-ammonia (specific gravity 0.88)-water (7:2:1, by volume). Tentative identification of GDP-fucose and fucose- $\text{PO}_4$  is based on chromatographic similarities to several sugar-nucleotides and sugar-phosphates in ethanol-1 M ammonium acetate (7:3, v/v; pH 7.5). The radioactivity of these components has been excluded from the calculations in Table 1.

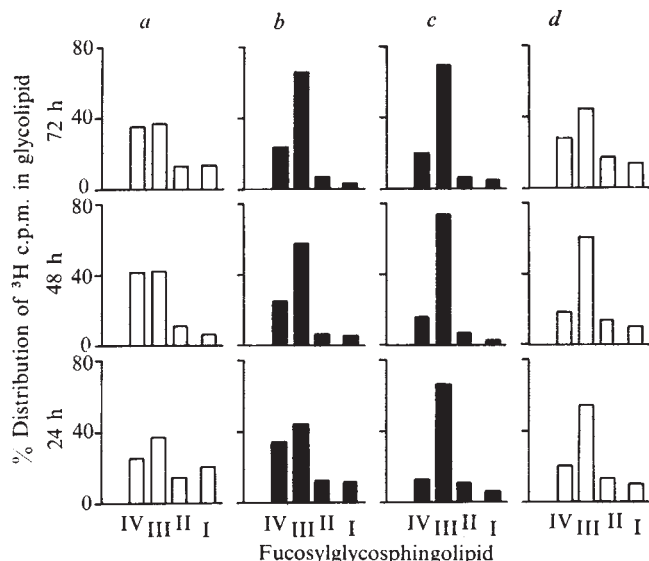


Fig. 2 Profiles of  $^3\text{H}$ -fucose-labelled glycolipids after reciprocal temperature shifts. NRK (MSV-1b) cells were grown at either  $39^\circ\text{C}$  (permissive (P) temperature) or  $33^\circ\text{C}$  (non-permissive (NP) temperature) for 72 h before addition of isotopically-labelled fucose to minimise possible cell density-dependent effects on glycolipid synthesis<sup>5</sup>. The medium was then removed and fresh medium containing  $1\text{-}^3\text{H}$ -L-fucose ( $5\text{ }\mu\text{Ci ml}^{-1}$ ,  $1.4\text{ Ci mmol}^{-1}$ , Amersham/Searle) was added. Half the cultures grown at  $33^\circ\text{C}$  (NP) were shifted to  $39^\circ\text{C}$  (NP  $\rightarrow$  P), while half of those at  $39^\circ\text{C}$  (P) were shifted to  $33^\circ\text{C}$  (P  $\rightarrow$  NP). At successive 24 h intervals thereafter, the cultures were examined morphologically and the fucosylglycolipids were analysed. Chromatography was carried out as in Fig. 1. The thin-layer plates were scraped and the major fucosylglycolipids (see Fig. 1) were counted by scintillation spectrometry. a, NP; b, NP  $\rightarrow$  P; c, P; d, P  $\rightarrow$  NP.

b) were comparable with cultures maintained at the permissive temperature. After 48 h many of the cells appeared morphologically normal and the fucosylglycolipid pattern resembled that of cultures maintained at the non-permissive temperature (that is, decrease in FucCer III, increase in FucCer I, II and IV). After 72 h essentially all the cells shifted to the non-permissive temperature appeared morphologically normal and the fucosylglycolipid pattern was comparable with that of cultures maintained continuously at the non-permissive temperature (Fig. 2a, 72 h) rather than that of cells maintained at the permissive temperature (Fig. 2c, 72 h).

These data show that alterations in fucosylglycolipid metabolism are related directly to the expression of the transformed state, and are not simply the result of MSV infection. The fucosylglycolipid pattern of NRK (MSV-1b) cells grown continuously at permissive temperature closely resembles the fucosylglycolipid pattern of cells transformed by the wild-type parent strain of MSV-MLV. In a parallel fashion, the fucosylglycolipid pattern of NRK (MSV-1b) cells grown continuously at non-permissive temperature resembles that of normal cells. In contrast, studies of nonfucose glycolipids (that is, gangliosides and their neutral glycolipid precursors) involving hamster cells infected with a temperature-sensitive (ts) transformation mutant of polyoma virus<sup>5</sup> or chick embryo cells with a ts transformation mutant of Rous sarcoma virus<sup>8</sup> have revealed similar non-fucose glycolipid patterns at both permissive and non-permissive temperatures. In addition, kinetic studies using the cold-sensitive NRK (MSV-1b) cells in temperature-shift experiments revealed a relationship between the morphology of the transformed cell phenotype and altered fucosylglycolipid synthesis. The relative rapidity of the fucosylglycolipid alterations after temperature-shift as compared with the stable pattern of the non-fucose glycolipids<sup>3,5</sup>, suggests a unique and dynamic role for fucosylglycolipids in transformation.

This work was supported by contracts within the Virus Cancer Program and grants from the National Cancer Institute and the Robert A. Welch Foundation. S.S. is an NIH special fellow. S.K. is a recipient of an NIH research career award.

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Received January 10; revised February 13, 1974.

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## Carbohydrate groups in the major glycoprotein of Rous sarcoma virus

GLYCOPROTEINS and other complex carbohydrates on the cell surfaces have been thought to be involved in many biological interactions<sup>1</sup>. Most enveloped viruses contain at least one glycoprotein component<sup>1</sup>. In avian sarcoma and leukaemia viruses some of the glycoproteins are located on the surfaces of the particles as projections composed of spikes and knobs<sup>2-4</sup>. Although their role is not well understood, the glycoproteins are believed to be involved in the determination of host range specificity, induction of neutralising antibody and interference capacity<sup>2,5-11</sup>. Rous sarcoma virus contains several glycoproteins<sup>10-13</sup>, and infectious and non-infectious strains of Rous sarcoma virus differ in major glycoprotein components<sup>14,15</sup>. We report here the results of purification of the major glycoprotein component (g2) of Rous sarcoma virus, its amino acid composition, and the nature of its carbohydrate groups.

The Bryan high-titre strain of Rous sarcoma virus RSV (RAV-1) was grown in type C/O or C/B chick embryo

TABLE 1 Amino acid composition of glycoprotein g2

| Amino acid | Mol % |
|------------|-------|
| Lys        | 3.66  |
| His        | 2.81  |
| Arg        | 4.47  |
| Asp        | 11.26 |
| Thr        | 7.56  |
| Ser        | 8.47  |
| Glu        | 11.70 |
| Pro        | 6.97  |
| Gly        | 9.33  |
| Ala        | 5.24  |
| Cys        | —     |
| Val        | 5.36  |
| Met        | 1.34  |
| Ileu       | 3.90  |
| Leu        | 8.33  |
| Tyr        | 4.51  |
| Phe        | 5.07  |

The glycoprotein, with added norleucine standard, was hydrolysed in 6 N HCl for 22 h at  $105^\circ\text{C}$ . Amino acid analysis was performed on a Technicon TSM amino acid autoanalyser.



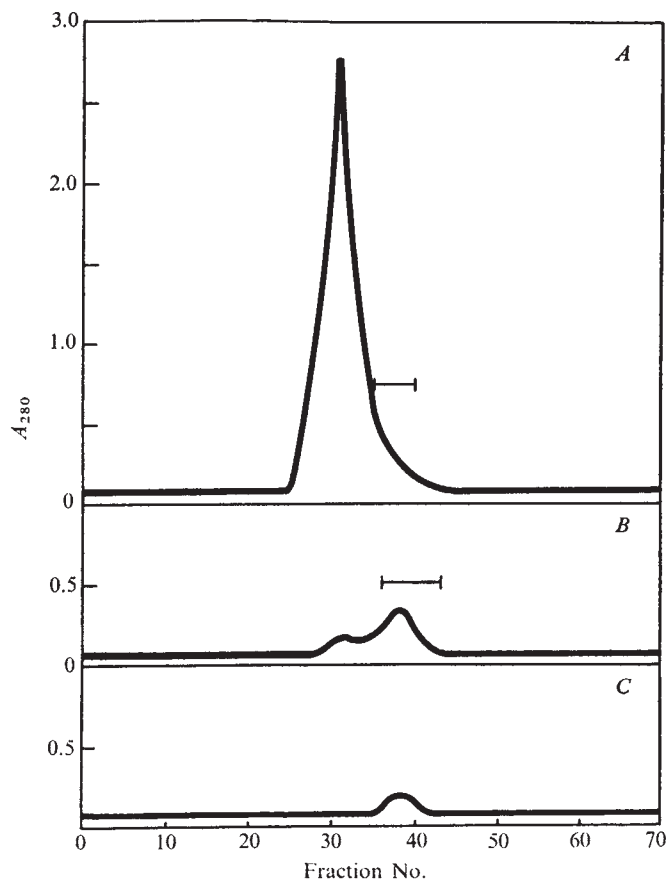


Fig. 1 Chromatography of viral proteins with Bio-Gel P-150. A, The phenol-extracted proteins of RSV(RAV-1) were passed through a column and fractions 35-40 (3.6 ml per fraction) were combined for rechromatography. B, The optical density profile after rechromatography of fractions 35-40 from (A). Fractions 36-43 were combined for further purification. C, Chromatography of the combined fraction from (B). Fractions 36-40 which contain homogeneous g2 glycoprotein by SDS-gel electrophoresis, were combined for the studies of amino acid and sugar compositions.

fibroblasts in an appropriate growth medium<sup>16</sup>. Large quantities of virus were isolated and purified from 40-60 l of culture medium by differential centrifugation and isopycnic banding as described before<sup>17</sup>. Viral protein was extracted with phenol from a preparation containing about 550  $A_{280}$  units of purified RSV(RAV-1) in the presence of sodium dodecylsulphate (SDS) and EDTA as reported earlier<sup>18</sup>. The viral protein was dissolved at 56°C in 0.1 M Tris buffer, pH 8.3 containing 6 M urea and 1% mercaptoethanol. Gel-filtration chromatography was carried out on a column of Bio-Gel P-150 (2.7 × 93 cm) in sodium phosphate buffer, 0.01 M, pH 7.2, and 0.1% mercaptoethanol. To locate glycoprotein g2, aliquots from the collected fractions were electrophoresed in SDS gels<sup>13</sup> and stained with Coomassie brilliant blue. The glycoprotein was detected in the trailing region, fractions 35-40 (Fig. 1A) of a peak near the void volume. Rechromatography of the trailing region on the same column showed two peaks (Fig. 1B). Rechromatography of fractions 36-43 (Fig. 1B) produced a peak containing only the glycoprotein g2 as analysed in SDS gel electrophoresis (Fig. 2). In a typical run, about 1.8  $A_{280}$  units of the protein were isolated.

Amino acid and sugar compositions (Tables 1 and 2) indicate that g2 is composed of 40% by weight of carbohydrate. A preparation containing 1.8  $A_{280}$  units contained 0.95 mg of peptide and 0.68 mg of carbohydrate. The minimal molecular weight calculated from the results of Tables 1 and 2 is about 12,500. From SDS-gel electrophoresis results, the

TABLE 2 Sugar composition of glycoprotein g2

| Sugar       | $\mu\text{mol per mg peptide}$ |
|-------------|--------------------------------|
| Glucosamine | 1.36                           |
| Mannose     | 1.20                           |
| Galactose   | 0.64                           |
| Fucose      | 0.10                           |
| Sialic acid | 0.40                           |

Hydrolysis conditions were as follows: for glucosamine, 4 N HCl for 6 h at 100°C; for neutral sugars, 2 N trifluoroacetic acid for 2 h at 100°C; for sialic acid, 0.05 N  $\text{H}_2\text{SO}_4$  for 1 h at 80°C. Amino sugars and neutral sugars were determined by automated assay as described<sup>19,20</sup>. Electronic expansion of the signal from the colorimeter by means of a linear converter (Dynacon Research and Systems, Paltisades, New York) facilitated determination of 0.001 to 0.02  $\mu\text{mol}$  of amino sugars. Sialic acid was assayed with autoanalytical chromatography (our work to be published) using the thiobarbituric acid reaction<sup>21</sup> to develop colour after elution of the sample from an anion exchange resin column. Each sugar analysis required about 25  $\mu\text{g}$  of glycoprotein, except that 150  $\mu\text{g}$  was used for the determination of fucose. All sugar measurements were made in the range of 5-15 nmol. No corrections were made for destruction of sugars during acid hydrolysis.

value of 75,000 had been assigned previously<sup>13</sup>. Although radioactive fucose has been shown to incorporate rapidly into glycoproteins of RSV<sup>10</sup>, chemical analysis shows a relatively small content of fucose.

Up to 45% of the sugar residues could be removed by digestion with glycosidases. Table 3 shows the release of sugars by  $\alpha$ -neuraminidase,  $\beta$ -galactosidase and  $\beta$ -N-acetylhexosaminidase, incubated separately and in combinations. All the sialic acid was released by neuraminidase.  $\beta$ -Galactosidase alone released 25% of the galactose, suggesting that some galactose residues are at terminal positions. After removal of sialic acid most remaining galactose residues were susceptible to galactosidase; about one additional mole was released per mole of sialic acid cleaved. Very little N-acetylglucosamine was released from intact g2 by  $\beta$ -N-acetylhexosaminidase. When neuraminidase and galactosidase were included in the incubation, nearly 50% of the N-acetylglucosamine was released—an amount about equal to the total galactose content of g2.

These results suggest that the structure of the terminal region of the carbohydrate unit consists largely of sequences: sialic acid  $\rightarrow$  galactose  $\rightarrow$  N-acetylglucosamine  $\rightarrow$ , and galactose  $\rightarrow$  N-acetylglucosamine  $\rightarrow$ . The same sequences have been found in many soluble vertebrate glycoproteins<sup>1</sup>. Recently, rabbit interferon was shown to have sialic acid  $\rightarrow$  galactose  $\rightarrow$  sequence<sup>23</sup>. The positions of mannose and the other glucosamine residues in the g2 glycoprotein have yet to be determined. The major glycoprotein described here corresponds to the knob projections in avian RNA tumour viruses<sup>2</sup>, and seems to perform an important function as it may recognise and interact with the receptor sites of

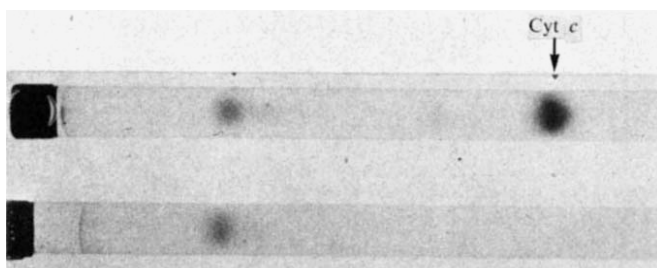


Fig. 2 Polyacrylamide gel electrophoresis of the purified glycoprotein from Bio-Gel P-150 chromatography. Aliquots of the combined fractions from Fig. 1C were electrophoresed alone (bottom) or together with horse heart cytochrome c (top) in SDS gels. The mobility of cytochrome c was about 3.4 times faster than that of the g2 glycoprotein.

TABLE 3 Glycosidase digestions of the g2 glycoprotein.

| Sugar measured | Enzyme treatment                                                                 | $\mu\text{mol}$ released<br>per mg peptide | %<br>Release |
|----------------|----------------------------------------------------------------------------------|--------------------------------------------|--------------|
| Sialic acid    | $\alpha$ -Neuraminidase                                                          | 0.43                                       | 107          |
| Galactose      | $\beta$ -Galactosidase                                                           | 0.15                                       | 23           |
|                | $\beta$ -Galactosidase +<br>$\alpha$ -Neuraminidase                              | 0.59                                       | 92           |
| Glucosamine    | $\beta$ -Hexosaminidase                                                          | 0.05                                       | 4            |
|                | $\beta$ -Hexosaminidase +<br>$\beta$ -Galactosidase +<br>$\alpha$ -Neuraminidase | 0.64                                       | 47           |

Neuraminidase (*C. perfringens*) was obtained from Boehringer Mannheim. Digestion was carried out overnight at 37°C in 0.03 M potassium acetate, pH 4.5, containing 0.03% bovine serum albumin (BSA), with an enzyme to substrate ratio of 1:15. Under these conditions, sialic acid was liberated completely from  $\alpha_1$ -acid glycoprotein. Aliquots of digestion mixture were assayed directly by the autoanalytical method (see Table 2). Jack bean  $\beta$ -galactosidase<sup>22</sup> was obtained from Dr Y. T. Li. Digestion was carried out at 23°C for 3 d in 0.03 M potassium acetate, pH 4.0, containing 0.03% BSA, a trace of thymol as preservative, and 1.5 enzyme units per 100  $\mu\text{g}$  of glycoprotein. Digestion was monitored at 24-h intervals by direct application of aliquots of mixture to the autoanalytical system. The enzyme remained active during the entire incubation period. Jack bean  $\beta$ -N-acetylhexosaminidase<sup>22</sup> was obtained from Dr Y. T. Li. Digestion was carried out at 23°C for 3 d in 0.06 M sodium citrate, pH 4.0, containing 0.02% BSA, a trace of thymol, and 25 units of enzyme per 100  $\mu\text{g}$  of glycoprotein. Digestion was monitored at 24-h intervals. The liberated N-acetylglucosamine was deproteinized with Dowex 50, deacetylated with 4 N HCl for 6 h at 100°C, and assayed as free glucosamine using the autoanalytical system. In experiments where combinations of enzymes were used, the incubation conditions for all enzymes were those appropriate for the first listed enzyme.

permissive host cells during the first step of a successful infection. Involvements of carbohydrate moieties during cellular interactions have been hypothesised<sup>24</sup>, and there is some supporting evidence<sup>25</sup>. The interplay between cell surface sialic acids and viral neuraminidase is now well understood<sup>26</sup>. It will be interesting to compare sugar sequences and amino acid compositions of glycoproteins from various strains of RSV. These glycoproteins are now being isolated and characterised.

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Received January 15, 1974.

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## Early changes in the kidneys of BALB/c mice infected with lactic dehydrogenase virus

Mice infected with lactic dehydrogenase virus (LDV) have lifelong viraemia and raised plasma enzyme levels<sup>1-4</sup>. In spite of rapid and prolonged virus replication, probably in the macrophages, only minor histological changes in lymphoid organs are reported<sup>5</sup>. Antibodies are produced<sup>6</sup> and complexes are said to be deposited in the glomerular capillary walls but only minimal glomerulonephritis results<sup>7,8</sup>.

Electron microscopic studies of liver, spleen and lymph nodes failed to show any changes, but in the kidneys of BALB/c mice there were striking changes as early as 24 h after infection, which persisted for at least 3 months. They were confined to the glomeruli and consisted of cytoplasmic endothelial swelling and darkly staining deposit between endothelial cells and basement membrane. There were no basement membrane changes or foot process adhesions. The electron micrograph (Fig. 1) of a glomerulus from an animal 12 h after infection shows markedly swollen endothelial cells filling the lumen of the glomerular capillaries. A dark exudate is present between many endothelial cells and the basement membranes, which are normal.

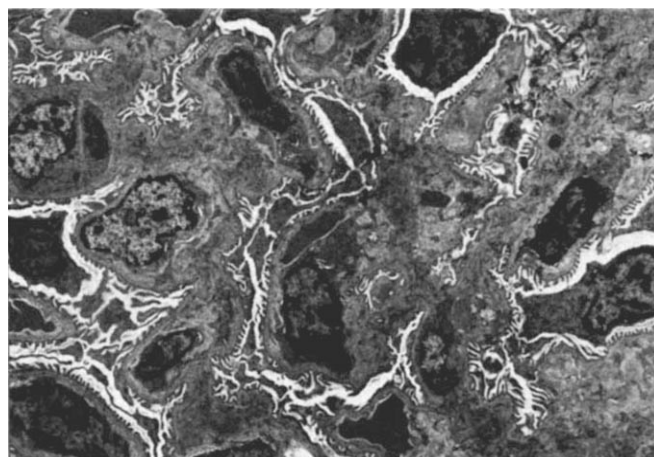


FIG. 1 Electron micrograph of a section of a glomerulus 12h after infection with LDV (X1,880).



TABLE 1 Effect of nephrectomy on replication of lactic dehydrogenase virus.

|                                    | Blood virus titre<br>(log <sub>10</sub> ID <sub>50</sub> ml <sup>-1</sup> )<br>4 h and 24 h after infection |     |
|------------------------------------|-------------------------------------------------------------------------------------------------------------|-----|
| Intact                             | 3.5                                                                                                         | 8.0 |
| Nephrectomised 2 h after infection |                                                                                                             | 3.0 |
| Nephrectomised 4 h after infection |                                                                                                             | 6.0 |

All the mice were injected intravenously with the same dose of virus and the figures given are the means of three titrations using two mice at each 10-fold dilution.

The early changes in the endothelial cells indicate that the virus might be replicating in them. To investigate this, the effect of bilateral nephrectomy on the level of infectivity of LDV 24 h after infection was measured. As shown in Table 1, total nephrectomy 2 h after infection reduced the level of viraemia at 24 h. The nephrectomised mice seem normal after the operation but by 24 h they were very ill. It was therefore likely that the reduced level of viraemia in the nephrectomised mice was due to toxæmia, rather than removal of a site of virus production. This view was supported by the observation that nephrectomy had less effect on virus levels if delayed for 4 h after infection, although no new virus enters the blood until 5 to 6 h after infection.

Unlike other authors<sup>7,8</sup> we have not found glomerular immune complex deposition in LDV-infected BALB/c mice in the first 3 months, so the changes in the kidneys seen by electron microscopy cannot be related to these deposits. It is possible that the glomerular lesions described here prevent the deposition of immune complexes in the glomeruli. Infection with LDV reduces the severity of spontaneous glomerulonephritis in New Zealand mice<sup>9</sup>. This may be due to reduced levels of antibody<sup>9</sup> but perhaps the changes produced in the glomerular capillary endothelium by the virus play some part. It would be interesting to know how infection with LDV would affect the development of nephritis resulting from chronic infection with lymphocytic choriomeningitis virus.

The study was in part supported by a grant from the Leukaemia Research Fund.

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## Reticulocyte DNA polymerase

DNA polymerase has been isolated from the cytoplasm of several mammalian tissues as well as several cell lines in culture<sup>1-8</sup>. This has been puzzling because DNA replication and repair are processes generally believed to be restricted to the nucleus, where chromatin is located. It has been suggested that cytoplasmic DNA polymerase is newly synthesised, not yet having been transported to the nucleus<sup>9</sup> or that it could have leaked from the nucleus during cell disruption<sup>10,11</sup>. Investigation of possible functions of a DNA polymerase in the cytoplasm have been inhibited by the possibility that the cytoplasmic location of this enzyme is either artefactual or trivial. To demonstrate that this is not so, we have investigated the presence of the enzyme in the reticulocyte, which is an anucleate cell. We wish to report here the purification and characterisation of a DNA polymerase from rabbit reticulocytes, and compare its properties with those of the DNA polymerase purified from the cytoplasm of immature erythroblasts of bone marrow, the precursor of the reticulocyte.

Reticulocytosis was induced by the procedure of Borsook *et al.*<sup>12</sup> New Zealand rabbits (4-6 pounds) were given four daily injections of 1.0 ml of 2.5% neutralised phenylhydrazine. Three days later they were bled by cardiac puncture. Whole blood contained more than 80% reticulocytes.

Washed reticulocytes were prepared by repeated suspension in 130 mM NaCl, 5.0 mM KCl and 7.4 mM MgCl<sub>2</sub>, centrifugation at 2,000 *g*, and removal of the buffy coat (nucleated cells and platelets). Washed reticulocyte preparations usually contained less than 0.5% nucleated cells, of which approximately 50% were immature red cells. Comparison of enzyme preparations from washed reticulocytes and from buffy coat cells indicated that enzyme activity is proportional to total cell number and not to the number of nucleated cells.

The packed reticulocytes from 400 ml of whole blood were lysed by addition of 250 ml lysis buffer (10 mM Tris-HCl, pH 7.4, 15 mM KCl and 5 mM 2-mercaptoethanol) followed by gentle stirring for 20 min at 0° C. Cell debris was removed by centrifugation at 30,000*g* for 15 min. The supernatant was made 5.0 mM in MgCl<sub>2</sub> and 0.5 M in KCl and the polysome fraction was removed by centrifugation at 78,000*g* for 120 min. The supernatant was made 40% saturated in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> by addition of solid (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> over 2 h. The (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> precipitate was collected by centrifugation at 30,000*g* for 15 min, suspended in TSED (50 mM Tris-HCl, pH 7.8, 1.0 mM dithiothreitol, 1.0 mM EDTA and 0.25 M sucrose), and dialysed against 1 l of TSED for 3 h with one change of buffer. The dialysed extract was applied to a phosphocellulose column (2.3 × 18 cm), previously equilibrated with TSED, and washed with the same buffer. A linear gradient of 0 to 1.0 M KCl in TSED with a combined volume of 300 ml was applied and 3 ml fractions were collected. DNA polymerase activity was eluted at 0.25 M KCl. The phosphocellulose fractions with activity were pooled and dialysed against 1 l of TGED (50 mM Tris-HCl, pH 7.8, 1.0 mM dithiothreitol, 1.0 mM EDTA, 25% glycerol) for 4 h with one change of buffer. The dialysed enzyme was applied to a DEAE-Sephadex A-50 column (1.8 × 9 cm), previously equilibrated with TGED, and washed with the same buffer. DNA polymerase was eluted stepwise with TGED containing 0.25 M KCl. A summary of the enzyme purification is shown in Table 1. As haem has been found to be a potent inhibitor of the enzyme, it is possible that the low activity of the crude enzyme fractions is due to inhibition by haem. The overall purification obtained was 2,400-fold with a yield of 73%.

In contrast to the cytoplasmic DNA polymerase of erythroblasts which is associated with the microsomes and

TABLE 1 Purification of DNA polymerase

| Step                                                            | Total Activity (U) | Protein (mg) | Specific Activity (U mg <sup>-1</sup> protein) | Fold-purification | % Yield |
|-----------------------------------------------------------------|--------------------|--------------|------------------------------------------------|-------------------|---------|
| 30,000g supernatant                                             | 706                | 28,125       | .025                                           | —                 | —       |
| 78,000g supernatant                                             | 585                | 24,750       | .024                                           | —                 | —       |
| 40% (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Precipitate | 1,000              | 1,280        | .788                                           | 31                | (100)   |
| Phosphocellulose chromatography                                 | 939                | 23.4         | 40.9                                           | 1,600             | (94)    |
| DEAE Sephadex chromatography                                    | 727                | 12.2         | 60.6                                           | 2,400             | (73)    |

The standard assay mixture contained in a final volume of 0.25 ml: 40 mM HEPES (N-2-hydroxyethylpiperazine-N'-2'-ethanesulphonic acid) buffer, pH 7.0, 0.4 mM MnCl<sub>2</sub>, 60 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.16 mM each dATP, dGTP and dCTP, 0.004 mM <sup>3</sup>H-dTTP, 2,000 μCi μmol, 20 μg activated calf thymus DNA and 5–10 μg enzyme. The reaction mixture was incubated for 15 min at 37°C and stopped by the addition of 2 ml of cold 5% trichloroacetic acid. The precipitate was collected on a glass fibre filter (Whatman GF/C) and washed with trichloroacetic acid and ethanol. The filter was dried and counted in a liquid scintillation counter. Activated calf thymus DNA was prepared as described by Aposhian and Kornberg<sup>18</sup>. One unit of activity is defined as the amount of enzyme which incorporates one picomole of dTMP in 15 min of incubation at 37°C.

is only solubilised in the presence of high salt concentrations, most of the reticulocyte enzyme is present in the cytosol even at low ionic strength.

The requirements for the synthesis of DNA are shown in Table 2. There is an absolute requirement for a template and a divalent cation. The rate of DNA synthesis is stimulated about three-fold by monovalent cation and about 10%

TABLE 2 Requirements for DNA synthesis

| Reaction conditions | <sup>3</sup> H-dTTP incorporated (pmol) | % Control |
|---------------------|-----------------------------------------|-----------|
| Complete            | 13.3                                    | 100       |
| —dithiothreitol     | 12.0                                    | 90        |
| —divalent cation    | 0.5                                     | 4         |
| —monovalent cation  | 4.0                                     | 30        |
| —template           | 0.4                                     | 3         |
| —enzyme             | —                                       | —         |
| —dATP               | 3.9                                     | 30        |
| —dATP, dCTP, dGTP   | 2.3                                     | 17        |

Incubation conditions were the same as described in Table 1 except that the individual components were omitted as indicated.

by dithiothreitol. All four deoxyribonucleoside triphosphates are required for optimal activity and in the absence of one or more deoxyribonucleoside triphosphates there is a substantial decrease in the rate of dTMP incorporation. This has also been observed with other mammalian DNA polymerases when one (or more) deoxyribonucleoside triphosphate was omitted<sup>5,14</sup>.

The divalent cation requirement can be satisfied by either Mn<sup>2+</sup> or Mg<sup>2+</sup>, although at different concentrations (Fig. 1),

TABLE 3 Template specificity

| Template                  | Concentration (μg per assay) | <sup>3</sup> H-dTTP incorporated (pmol) |
|---------------------------|------------------------------|-----------------------------------------|
| None                      | —                            | —                                       |
| Activated calf thymus DNA | 20                           | 15.2                                    |
| Native calf thymus DNA    | 20                           | 3.8                                     |
| T4 DNA                    | 10                           | 0.8                                     |
| Hb mRNA                   | 10                           | —                                       |
| rRNA                      | 10                           | —                                       |
| Yeast tRNA                | 10                           | —                                       |
| poly d(A-T)               | 4                            | 6.0                                     |
| poly (dA)·poly (dT)       | 2                            | 1.1                                     |
| poly (rA)·poly (dT)       | 5                            | —                                       |
| poly (rA)·oligo (dT)      | 10                           | —                                       |
| poly (dA)                 | 2                            | 0.8                                     |
| poly (A)·poly (U)         | 10                           | 0.2                                     |
| poly (A)                  | 10                           | 0.2                                     |

Reaction conditions were as described in Table 1 except for the addition of templates as indicated.

the optimal concentration of MgCl<sub>2</sub> being ten times that of MnCl<sub>2</sub>. The effect of monovalent cation concentration is shown in Fig. 2. Although all the monovalent cations tested stimulate the rate of DNA synthesis, KCl gives the greatest stimulation, and NH<sub>4</sub>Cl and NaCl are less effective. The optimal concentration for all three monovalent cations is 100 mM at pH 7.0. Both the reticulocyte enzyme and the erythroblast enzymes can use either Mn<sup>2+</sup> or Mg<sup>2+</sup> as divalent cation and both are stimulated optimally by 100 mM KCl.

At optimal conditions of pH and ionic strength, the reticulocyte DNA polymerase sediments predominantly at 8S, with a smaller peak of activity at 11S (Fig. 3). This is also the case with the erythroblast enzyme, which has been shown to dissociate from an 11S dimer to an 8S monomer under conditions of salt activation<sup>7</sup>. No DNA polymerase activity sedimenting at 3.4S has been detected. Chang and Bollum have found a high molecular weight DNA polymerase of 6–8S and a low molecular weight DNA polymerase of 3.4S in the cytoplasm of bone marrow and several other tissues, while only the 3.4S DNA polymerase

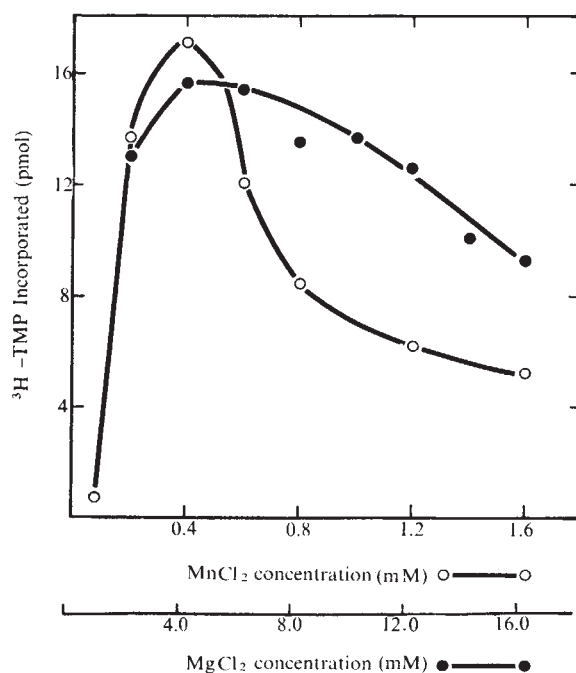


FIG. 1 Effect of divalent cations. Assay conditions were as in Table 1 except for the concentrations of divalent cations. MgCl<sub>2</sub> (●) and MnCl<sub>2</sub> (○).



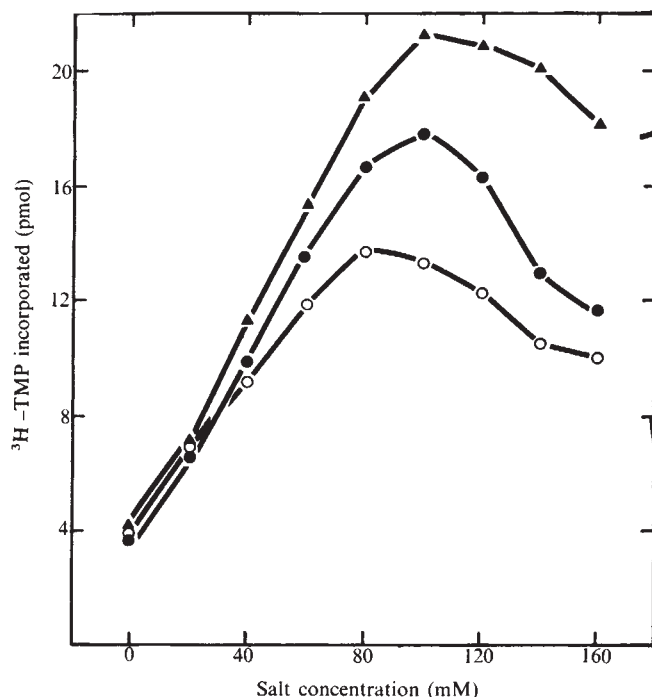


FIG. 2 Effect of monovalent cations. Reaction conditions were as in Table 1 except for the salt concentration. NH<sub>4</sub>Cl (●), KCl (▲) and NaCl (○).

was present in the nuclei of these tissues<sup>5,15</sup>. The absence of a 3.4S DNA polymerase in reticulocytes is consistent with their lack of a nucleus.

The ability of several DNAs, RNAs and synthetic polynucleotides to serve as templates for the reticulocyte DNA polymerase was tested (Table 3). As is the case with the erythroblast cytoplasmic DNA polymerase, activated calf thymus DNA was the best template for the reticulocyte enzymes. However, in contrast to the erythroblast enzyme, poly d(A-T) the alternating copolymer of deoxyadenylate and deoxythymidylate, was less than half as active as activated calf thymus DNA<sup>7</sup>. Double stranded synthetic DNAs such as poly(dA)·poly(dT), or DNA·RNA hybrids were either inactive or very poor templates, as were single stranded RNAs or single stranded homopolymers such as poly(dA) or poly(A). Native calf thymus DNA was less effective as a template than activated calf thymus DNA, suggesting that the reticulocyte enzyme, as well as the erythroblast enzyme, cannot initiate DNA synthesis in the absence of a primer.

Similar to the erythroblast enzyme<sup>7</sup>, the reticulocyte DNA polymerase is markedly inhibited by the rifamycin derivative AF/013 which has also been shown to inhibit the nuclear DNA-dependent RNA polymerases<sup>16-18</sup> as well as the viral RNA-dependent DNA polymerase<sup>18</sup>. The synthesis of DNA is also very sensitive to the intercalating dye ethidium bromide, being 50% inhibited at 8  $\mu$ g ml<sup>-1</sup>. Of particular interest is the inhibitory effect of haem which has been implicated in the control of haemoglobin synthesis in reticulocytes at the translational level<sup>19,20</sup>. The concentration of haem that completely inhibits the cytoplasmic DNA polymerase (10<sup>-5</sup> M) is the same as that reported to stimulate globin synthesis optimally<sup>21</sup>.

As with the erythroblast cytoplasmic DNA polymerase<sup>8</sup>, the synthesis of DNA with the reticulocyte enzyme is stimulated by ribonucleoside and deoxyribonucleoside triphosphates. The greatest degree of stimulation is seen with ATP which stimulates the rate of DNA synthesis 3.5-fold, while GTP, UTP and CTP stimulate 2.5 to 3-fold. The  $\alpha$ ,  $\beta$ - and  $\beta$ ,  $\gamma$ -methylene analogues of ATP are also stimulatory, as is ADP and  $\alpha$ ,  $\beta$ -methylene ADP. However, 3'-AMP and

5'-AMP have no effect on the reaction. We have previously shown that the erythroblast cytoplasmic DNA polymerase is activated by ribonucleoside diphosphates and triphosphates and that the effect of these activators is to increase the  $V_{max}$  of the enzyme rather than to lower the  $K_m$  values for the substrates<sup>8</sup>. Furthermore, it has been shown that the mechanism of ATP activation involves the dissociation of the enzyme from an inactive 11.6S dimer to an active 8S monomer. Presumably the reticulocyte enzyme responds to nucleotide activation in the same way. The enzyme isolated from reticulocytes is very similar in its properties to that isolated from the cytoplasm of the nucleated erythroblasts of bone marrow; one difference being that most of the reticulocyte enzyme is not associated with the microsomes even at low ionic strength.

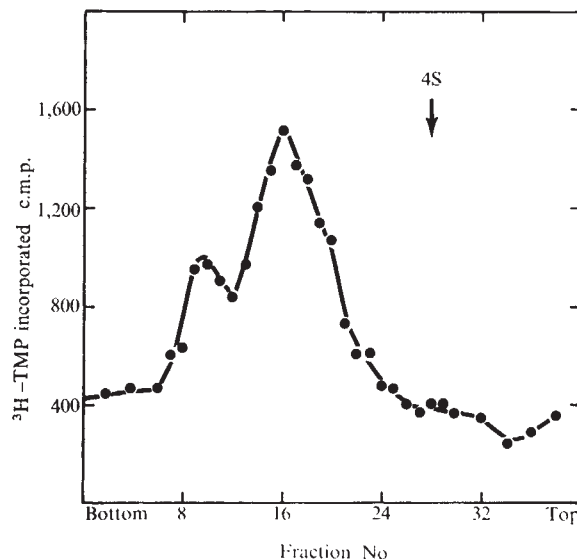


FIG. 3 Sucrose density gradient analysis of reticulocyte DNA polymerase. DNA polymerase was dialysed for 4h against 5% sucrose buffer containing 10 mM HEPES buffer, pH 7, 1.6 mM MnCl<sub>2</sub>, 1.0 mM dithiothreitol and 40 mM KCl. A sample of 0.2 ml of enzyme solution containing 320  $\mu$ g of protein was layered on a 5-20% sucrose gradient and sedimentation was carried out in a Spinco SW 39 rotor for 15 h at 37,000 r.p.m. at 2°-4° C. Simultaneously 4S tRNA was sedimented as a marker. Thirty-eight equal fractions were collected from the bottom of the tubes and assayed for DNA polymerase activity.

The purification of DNA polymerase from reticulocytes is good evidence that this is in fact a cytoplasmic enzyme. However, the exact role of the cytoplasmic DNA polymerase is not known. It has been proposed that this enzyme is involved in processes of cellular differentiation and gene amplification<sup>7</sup>. This is particularly relevant in an erythroid cell where a large quantity of haemoglobin is synthesised in a relatively short time and no evidence of nuclear gene amplification for this protein has been demonstrated<sup>22,23</sup>. It is our hypothesis that cytoplasmic DNA and RNA polymerases are involved in the regulation of expression and amplification of genetic information<sup>7,24</sup>.

The research was supported by grants from the National Institutes of Health, American Heart Association and the Milton N. Weir, jr Cancer Research Fund. A. G. S. holds an established investigatorship from the American Heart Association.

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Received November 6, 1973; revised January 28, 1974.

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22° C, the cells were washed three times by centrifugation. BALB/c female mice were inoculated i.p. with a single injection of  $5 \times 10^7$  glutaraldehyde-treated cells. Age matched non-immunised mice were kept as controls. After 2 weeks, both groups were challenged i.p. with live tumour cells. Cages containing the different groups were coded before challenge so that the operator could not identify the groups during injection or subsequent inspections.

Non-immunised control mice (Table 1) challenged with  $10^5$  tumour cells survived for approximately 20 d. Immunised mice were completely protected against this challenge (experiments I, II, III, IV) but not against challenge by  $10^6$  tumour cells (experiments I and III). In contrast, mice immunised with the same number of irradiated cells showed less protection (experiment IV). In one experiment (II) the glutaraldehyde-treated cells were stored for 2 weeks at 4° C before immunisation. These cells gave similar full protection against the challenge. Mice left for 27 d between immunisation and challenge showed only partial protection (experiment V), indicating that immunity was decreasing at that time.

Preliminary data with a second tumour (a mammary tumour which arose spontaneously in our colony) corroborates these findings. Furthermore, we have found that protection against a higher challenge dose can be achieved with multiple immunisation, whereas mice injected with a similar number of glutaraldehyde-treated syngeneic spleen cells showed no protection. This data will be presented in a subsequent publication.

Two factors might be operating in producing this immunity. First, glutaraldehyde may be preserving the tumour antigenicity and, second, the chemical modification of the proteins may be producing an enhanced cellular immunity at the expense of the humoral response. This phenomenon has been studied in some detail with protein antigens<sup>6,7</sup>. The implication is that the development of enhancing antibody may be minimal. Further experiments are in progress to study the mechanisms involved.

## The induction of tumour immunity in mice using glutaraldehyde-treated tumour cells

INDUCING immunity to tumours presents problems similar to those of inducing immunity to infectious microorganisms—the presentation of the agent in an antigenic but non-infectious form. Two approaches have been used to render syngeneic tumour cells non-infectious, lethal irradiation<sup>1</sup> and treatment with chemically reactive groups such as iodoacetamide<sup>2,3</sup>. These treatments leave the cell metabolising but incapable of division.

Here we demonstrate the use of glutaraldehyde-treated cells for the induction of immunity in syngeneic hosts. Glutaraldehyde solution contains polymeric  $\alpha$  and  $\beta$  unsaturated aldehydes resulting from aldol condensation. These react rapidly with protein amino groups in mild conditions and cross link the protein molecules. The modification is irreversible and does not disorder the crystal structure<sup>4</sup>. For these reasons glutaraldehyde has found wide application as a fixative for electron microscopy and X-ray diffraction.

A methyl-cholanthrene-induced BALB/c tumour (Meth A)<sup>5</sup> was passaged in BALB/c mice by intraperitoneal (i.p.) inoculation. This transplantable tumour is highly infectious in syngeneic animals and a dose as low as  $10^3$  i.p. will cause ascites and eventual death of the animals. Cells collected from the peritoneum were used for immunisation and challenge. Cells for immunisation were washed in phosphate-buffered saline (PBS), pH 7.0, and made up to  $10^6$  ml<sup>-1</sup>. An equal volume of 0.25% glutaraldehyde (Taab Laboratories, Reading, England) in PBS was added. After 10 to 15 min at

TABLE 1 The protective effect of glutaraldehyde-treated tumour cells on subsequent challenge with live tumour cells.

| Immunisation dose of glutaraldehyde-treated tumour cells | Time before challenge (d) | Challenge dose of live tumour cells (i.p.) | Survivals* |    |    |    |  |
|----------------------------------------------------------|---------------------------|--------------------------------------------|------------|----|----|----|--|
|                                                          |                           |                                            | Day 0      | 10 | 20 | 30 |  |
| Experiment I                                             |                           |                                            |            |    |    |    |  |
| $5 \times 10^7$                                          | 14                        | $10^5$                                     | 5          | 5  | 5  | 5† |  |
| Control                                                  |                           | $10^5$                                     | 5          | 5  | 2  | 0  |  |
| Experiment II                                            |                           |                                            |            |    |    |    |  |
| $5 \times 10^7$ (stored cells)‡                          | 14                        | $10^5$                                     | 5          | 5  | 5  | 5  |  |
| Control                                                  |                           | $10^5$                                     | 5          | 5  | 0  | 0  |  |
| Experiment III                                           |                           |                                            |            |    |    |    |  |
| $5 \times 10^7$                                          | 14                        | $10^5$                                     | 5          | 5  | 5  | 5  |  |
|                                                          |                           | $10^6$                                     | 5          | 5  | 0  | 0  |  |
| Control                                                  |                           | $10^5$                                     | 5          | 5  | 1  | 0  |  |
|                                                          |                           | $10^6$                                     | 5          | 5  | 0  | 0  |  |
| Experiment IV                                            |                           |                                            |            |    |    |    |  |
| $5 \times 10^7$ (irradiated cells)§                      | 14                        | $10^5$                                     | 5          | 5  | 3  | 1  |  |
| $5 \times 10^7$                                          | 14                        | $10^5$                                     | 5          | 5  | 5  | 5  |  |
| Control                                                  |                           | $10^5$                                     | 5          | 5  | 0  | 0  |  |
| Experiment V                                             |                           |                                            |            |    |    |    |  |
| $5 \times 10^7$                                          | 27                        | $10^5$                                     | 4          | 4  | 2  | 2  |  |
| Control                                                  |                           | $10^5$                                     | 5          | 4  | 3  | 0  |  |

\* Mice developing tumours were usually recognisable before day 10, but only deaths are recorded in this table.

† Surviving mice were observed up to 60 d. No further tumours developed. This group was re-challenged on day 57 with  $10^6$  tumour cells i.p. None survived.

‡ Treated cells stored for 2 weeks at 4° before immunisation.

§ Cells were washed and resuspended at  $5 \times 10^7$  ml<sup>-1</sup> in PBS immediately before subjecting to 4000 rads  $\gamma$  irradiation from a <sup>60</sup>Co source.



These preliminary results show that tumour immunity can be induced using glutaraldehyde-treated tumour cells. Apart from the possibly greater immunogenicity, compared with irradiated cells, the treatment is simple and the cells are non-viable. Furthermore, it may be possible to store the treated cells for repeated immunisation. By comparison with previously published data<sup>2,3,8</sup>, the high degree of protection achieved is sufficiently encouraging to justify experiments with other tumours and other species. If the efficacy can be substantiated the procedure could have advantages in the investigation of tumour immunotherapy in man.

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## Fluctuations and distribution of measles virus antigens in chronically infected cells

THE question of chronic measles virus infections in human central nervous system disease has become increasingly important. In subacute sclerosing panencephalitis the role of measles virus has been established<sup>1</sup>. In multiple sclerosis the role of measles is less clear although seemingly implicated<sup>2</sup>. It has been suggested that a virus-induced immunopathological process may be involved, initiated by the incorporation of viral antigens into the surface membrane of cells<sup>3</sup>. The study of measles envelope antigens on the surface of cells in an *in vitro* model is therefore of interest. Here we report the quantitative fluctuations of measles virus antigens at the cell surface in chronically infected cell

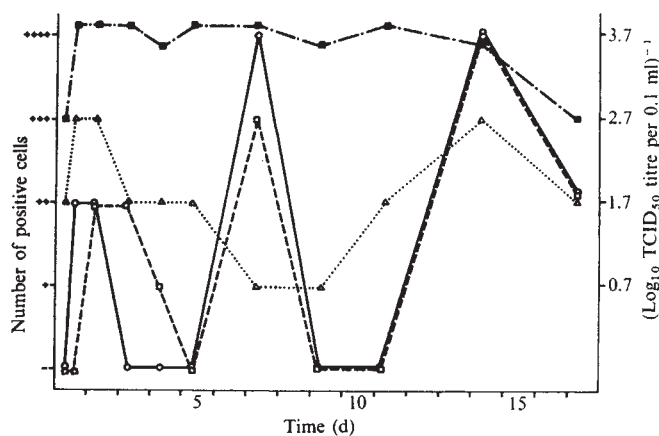


FIG. 1 Fluctuations of membrane immunofluorescence, haemadsorption and extracellular infectivity. ○—○, Immunofluorescence with anti-HL serum; □—□, immunofluorescence with anti-HA serum; ■—■, haemadsorption; △···△, infectivity TCID<sub>50</sub> titres.

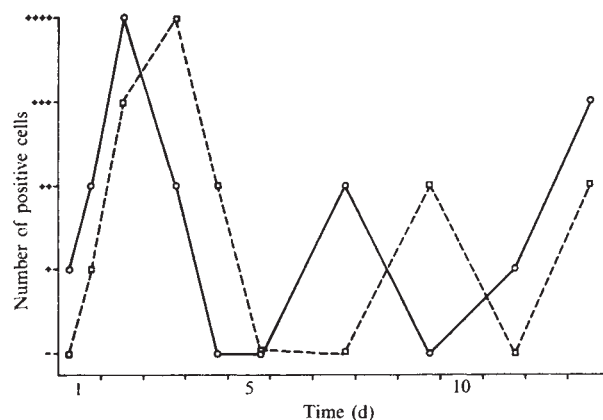


FIG. 2 Fluctuations of surface antigen expression in cells chronically infected with measles virus. Membrane immunofluorescence specific for HL (○) and HA (□).

cultures measured by haemadsorption and indirect membrane immunofluorescence.

A carrier cell line of the Edmonston strain of measles virus in a human heteroploid lung cell line (Lu 106) was used<sup>4</sup>. The cells were maintained for 24 h in Eagle's minimum essential medium (E-MEM) containing 1% L-glutamine, penicillin and streptomycin and 1% calf serum to synchronise cell division before the initiation of individual experiments. Vero cells lytically infected with measles virus were used as controls to test the reliability of the serum, conjugate, and staining technique. Cells were trypsinised in 0.1% trypsin–0.02% versene buffer and suspended in 2% calf serum in E-MEM and 30,000 cells ml<sup>-1</sup> were placed on coverslips (10 × 40 mm) within sealed Leighton tubes at 37° C. Uninfected Lu 106 cells were used as controls. The cell cultures approached a monolayer by the end of two weeks. Coverslips were taken for examination every 4 h for the first 12 h and at 24 h intervals for the first 4 d and then on three alternate days each week. A parallel series of extracellular samples were collected for determination of infectivity titres in roller tubes of Vero cells. From serial ten-fold dilutions 0.1 ml samples were taken and inoculated into three tubes per dilution. Final readings were made 14 d after inoculation and the tissue culture infectious dose causing specific cytopathic effects in 50% of the tubes (TCID<sub>50</sub>) was calculated. Haemadsorption was carried out by washing with phosphate-buffered physiological saline (PBS) without Mg<sup>2+</sup> and Ca<sup>2+</sup> and addition of 0.1% African green monkey cells. After adsorption at room temperature for 1 h the coverslips were washed twice with PBS and then examined by light microscopy.

Antisera were prepared by hyperimmunising rabbits (using Freund's complete adjuvant and an intravenous booster after 5 week) with purified whole virus from Vero cells, small particle haemagglutinin (HA) (ref. 5), and virus particles from which surface projections had been removed by treatment with 0.004% trypsin. This treatment removes HA projections but leaves some envelope-associated haemolysin (HL) (Norrby, to be published). Sera were tested by haemagglutination inhibition (HI) and haemolysin inhibition (HLI) tests as described previously<sup>6</sup>. Anti-HA serum had an HI antibody titre of 5,000, but no HLI antibodies demonstrable after removal of HI antibodies by absorption with Tween 80-ether antigen<sup>6</sup>. Anti-HL serum had an HLI titre of 320 and an HI titre of less than 5. Rabbit control serum against uninfected Vero cells was prepared. Before use in immunofluorescence measurements sera were absorbed with non-infected Vero cells.

For membrane immunofluorescence measurements coverslips were washed twice in PBS without Ca<sup>2+</sup> and Mg<sup>2+</sup> and incubated with specific antiserum for 30 min at room

temperature. Antiserum was removed by washing twice in PBS; sheep anti-rabbit gamma globulin conjugated to fluorescein isothiocyanate (FITC) (National Bacteriological Laboratory, Stockholm, Sweden) was added for 30 min. After two additional washings in PBS, coverslips were mounted in buffered glycerol on glass slides. The slides were examined immediately with a Zeiss fluorescence microscope. After storage at 4° C a second blind reading was performed by another person at the end of the experiment.

The number of cells showing intense fluorescence was estimated. A grading from + to ++++ was used; + representing 10–25%, ++ 25–50%, +++ 50–75%, ++++ more than 75%. Periodic fluctuations in the numbers of cells displaying membrane HA occurred in all four experiments (Figs 1 and 2). During the first 2–4 d the number of positive cells increased to a maximum of ++ to ++++. Thereafter fluorescence disappeared and remained absent for 1–2 d. Fluorescence then reappeared independently of growth medium changes. During the 2 week observation period one more cycle of disappearance and reappearance was encountered in three experiments.

Membrane fluorescence specific for HL antigen fluctuated in two experiments (Figs 1 and 2). It remained absent throughout the other two experiments despite variations of the HA antigen. HL antigen expression was occasionally found in the absence of HA antigen (Fig. 2).

The titre of extracellular infectious virus varied but did not show a distinct correlation with presence or absence of membrane antigens as measured by immunofluorescence (Fig. 1). Intracellular antigen and haemadsorption fluctuated only a little and never occurred in fewer than 75% of cells. Haemadsorption therefore was a more sensitive indicator of surface measles antigen than membrane immunofluorescence.

A polar distribution of antigen (capping) commonly occurred in single cells or in cells appearing to undergo division (Fig. 3*a* and *b*). In Fig. 3*b* there is polar fluorescence at opposite ends of what may be a cell in mitosis. In other cells the discontinuous membrane immunofluorescence was most common (Fig. 4*a* and *b*). The relatively smaller syncytium in *a* displays a more intense membrane immunofluorescence than the syncytium in *b*. This was generally true.

Several membrane antigens have the capacity to redistribute<sup>7–9</sup>. Marcus found viral protein at one end of cells by haemadsorption in NDV-infected cells<sup>10</sup>. Capping of viral antigen might be of some interest with reference to a possible occurrence of antigenic modulation<sup>9</sup>.

Various factors such as cell growth<sup>11,12</sup>, phases of the cell cycle<sup>11,12</sup>, energy generating system<sup>13</sup> and pinocytosis of antigen-antibody complexes after capping<sup>9</sup>, have been

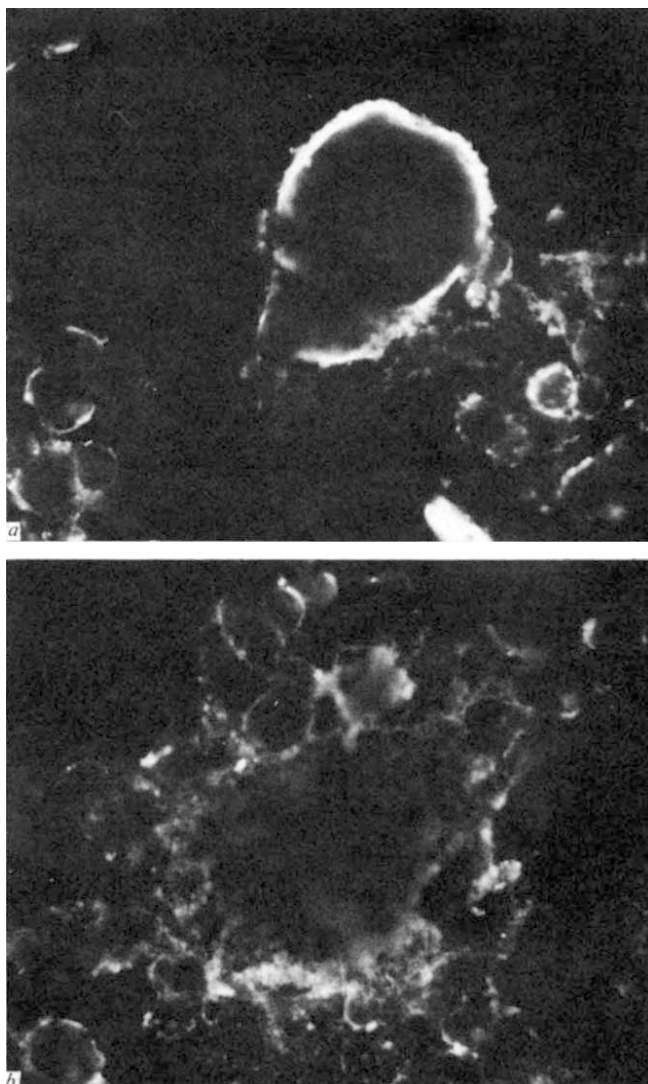


FIG. 4 Membrane antigens on single cells and syncytia in a culture chronically infected with measles virus, demonstrated by the indirect immunofluorescence technique using an anti-whole virus serum. Magnification  $\times 750$ .

found to cause fluctuating expression of different surface antigens. In a cell line chronically infected with virus, factors such as autointerference or interferon production<sup>14,15</sup> may also be of importance. It has been observed (A. E., to be published) that carrier Lu 106 cells display a varying sensitivity to measles virus-specific cytotoxic antibodies. This may relate to the variable expression of membrane antigen as shown by immunofluorescence.

In multiple sclerosis the pathogenesis and aetiology of the disease is not known, but it is possible that it represents a chronic infection and that the exacerbations and remissions characteristic of the disease are the result of irregular expression of virus-specific antigens in myelin membranes and an immunological attack on these antigens.

This work was supported by a grant from the Swedish Medical Research Council.

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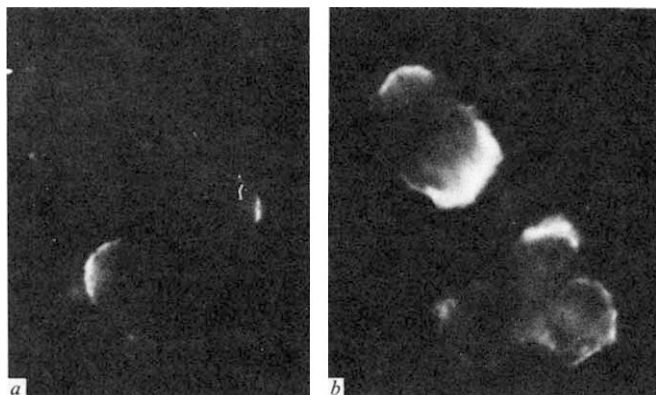


FIG. 3 *a* and *b*, Capping of measles virus HA antigen demonstrated by indirect membrane immunofluorescence. Magnification  $\times 930$ .



Received November 23, 1973; revised January 23, 1974.

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## Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans

CHANGES in brain tryptophan (Trp) associated with feeding are relevant to serotonin (5-hydroxytryptamine, 5-HT)-mediated function, because in the brain Trp levels modulate 5-HT synthesis<sup>1</sup>. Using rodents, some investigators<sup>2-5</sup> have confirmed our observations<sup>6,7</sup> that after ingestion of balanced diets plasma Trp increases but brain Trp decreases or is unchanged. Other such dissociations between plasma and brain Trp have been observed during fasting, and after eating in hypophysectomised rats, indicating that the changes are not secondary to stress or pituitary mechanisms<sup>7</sup>. The dissociation is also independent of diurnal or nocturnal variations in plasma Trp<sup>4,7</sup>, but there is no explanation for it.

On the other hand, ingestion of pure carbohydrate (CHO) diets<sup>8-10</sup>, and injection of Trp<sup>5</sup> and drugs that affect 5-HT metabolism<sup>11</sup> increase both total plasma and brain Trp. This

led to the hypothesis that levels of total plasma Trp reflect levels of brain Trp<sup>8</sup>. The hypothesis has been challenged by evidence that injections of Trp increase free plasma much more than total plasma Trp<sup>5</sup>.

A further challenge is the report that CHO diets increase total plasma and brain Trp, but decrease free plasma Trp<sup>10</sup>. Some drugs that increase brain Trp and 5-HT metabolism do not increase free plasma Trp<sup>12</sup>. Furthermore, transport of Trp from plasma to brain depends on levels of competing plasma amino acids<sup>9</sup> and on the ratio between total plasma Trp and neutral amino acids (NAA) in plasma<sup>9</sup>. So far, there is general agreement that after ingestion of balanced diets<sup>2,3,5</sup> or CHO diets<sup>10,13</sup>, free plasma Trp is decreased, and total and bound plasma Trp are increased.

Tryptophan is an essential amino acid, normally binding to albumin in plasma<sup>14</sup>. Displacement of Trp from the binding site in the albumin molecule by free fatty acids (NEFA)<sup>2,3,10</sup>, and by drugs that decrease plasma Trp but increase brain Trp<sup>11,15</sup>, is a suggested cause of plasma-brain Trp dissociations. It has been speculated that CHO and insulin increase the albumin-binding of Trp by decreasing the extent of saturation of albumin with NEFA<sup>10</sup>. Decreases of plasma NEFA after ingestion of balanced diets or insulin and CHO diets are well known<sup>16</sup>. Mobilisation of NEFA can also be influenced by concentrations of plasma triglycerides of hepatic or dietary origin<sup>16</sup>. A role of NEFA in controlling food intake has been postulated<sup>17</sup>, but whether plasma Trp (free or bound) is concerned with the control of food intake remains to be seen. In humans, injection of insulin increases total plasma Trp and decreases plasma NAA<sup>18</sup>, and CHO ingestion reduces plasma NEFA and free plasma Trp<sup>13</sup>.

We have investigated the mechanisms mediating plasma-brain Trp dissociations and looked for similar relationships between total, bound and free plasma Trp and cerebrospinal fluid (CSF) in human subjects after intake of a balanced diet.

Twenty-nine subjects with Parkinson's disease, Huntington's chorea or related neurological disorders volunteered for diagnostic studies of monoamine metabolism. They were fasting and recumbent in bed for 12 h before CSF was drawn by lumbar puncture (at 1000 h), and samples were mixed with 2% ascorbic acid and cooled to -20°C. Blood was drawn by venipuncture and centrifuged. The plasma was stored at -20°C. This procedure was repeated at 1600 h. Between sampling all subjects remained in bed; nine fasted all the

TABLE 1 Total, bound and free plasma Trp, CSF-Trp and CSF-5-HIAA in man during fasting and after feeding

| Conditions               | Total<br>μg ml <sup>-1</sup> ± s.e.m. | Plasma Trp<br>Bound<br>μg ml <sup>-1</sup> ± s.e.m. | Free<br>μg ml <sup>-1</sup> ± s.e.m. | CSF-Trp<br>ng ml <sup>-1</sup> ± s.e.m. | CSF-5-HIAA<br>ng ml <sup>-1</sup> ± s.e.m. |
|--------------------------|---------------------------------------|-----------------------------------------------------|--------------------------------------|-----------------------------------------|--------------------------------------------|
| Diet a                   |                                       |                                                     |                                      |                                         |                                            |
| Fasting 12 h<br>(1000 h) | 10.95 ± 0.87(14)                      | 9.38 ± 1.14(7)                                      | 0.55 ± 0.09(7)                       | 377.30 ± 41.40(14)                      | 49.20 ± 4.80(14)                           |
| Postprandial<br>(1600 h) | 14.44 ± 1.01(14)                      | 12.36 ± 0.38(7)                                     | 0.20 ± 0.06(7)                       | 303.90 ± 31.00(14)                      | 39.60 ± 3.30(14)                           |
| % Change                 | 32%****                               | 32%****                                             | -64%****                             | -20%****                                | -20%***                                    |
| Diet b                   |                                       |                                                     |                                      |                                         |                                            |
| Fasting 12 h<br>(1000 h) | 12.08 ± 0.38(6)                       | 8.92 ± 0.19(6)                                      | 0.49 ± 0.02(6)                       | 269.00 ± 10.44(6)                       | 27.18 ± 3.79(6)                            |
| Postprandial<br>(1600 h) | 17.25 ± 1.54(6)                       | 14.99 ± 1.52(6)                                     | 0.35 ± 0.02(6)                       | 211.66 ± 17.41(6)                       | 17.63 ± 3.68(6)                            |
| % Change                 | 43%****                               | 68%****                                             | -29%***                              | -21%*                                   | -35%*                                      |

Total plasma Trp was isolated<sup>19</sup> and bound and free (ultrafiltrate) plasma Trp were separated as before<sup>20</sup>. CSF-Trp and the main metabolite of 5-HT in CSF, 5-hydroxyindoleacetic acid (5-HIAA), were separated in a Dowex 50, Na<sup>+</sup> resin<sup>21</sup>.

Plasma NAA (valine, isoleucine, leucine, tyrosine and phenylalanine) were separated by column chromatography with a Beckman model 120 C, amino acid analyzer. The plasma ratios between Trp (total, bound or free) and NAA were obtained by dividing Trp in plasma by the sum of the five plasma NAA<sup>9</sup>. Trp and 5-HIAA were determined fluorometrically as described elsewhere<sup>7,11</sup>. Total serum protein, albumin, and triglycerides were determined by the clinical laboratory of NIH. Statistical analyses were done using paired Student's *t* tests and linear correlations<sup>22</sup>.

Numbers in parenthesis are number of subjects.

s.e.m., standard error of the mean<sup>22</sup>.

Statistical significance, \* *P* < 0.05, \*\* *P* < 0.02, \*\*\* *P* < 0.01, \*\*\*\* *P* < 0.001.

% Change, represents the percentage change between fasting and postprandial levels.

TABLE 2 Plasma levels of neutral amino acids, Trp, and ratios of total and free plasma Trp to neutral amino acids, before and after feeding

| (A)                      |  | 12 h fasting (1000 h)                   | Postprandial (1600 h)                   |
|--------------------------|--|-----------------------------------------|-----------------------------------------|
| Plasma amino acids (NAA) |  | $\mu\text{g ml}^{-1} \pm \text{s.e.m.}$ | $\mu\text{g ml}^{-1} \pm \text{s.e.m.}$ |
| Valine (V)               |  | $21.7 \pm 2.0$                          | $28.8 \pm 2.0$ (32.7%)*                 |
| Isoleucine (I)           |  | $7.3 \pm 0.9$                           | $11.8 \pm 1.1$ (61.6%)**                |
| Leucine (L)              |  | $11.2 \pm 1.0$                          | $17.9 \pm 1.4$ (59.8%)**                |
| Tyrosine (T)             |  | $7.9 \pm 0.6$                           | $11.4 \pm 0.6$ (44.3%)**                |
| Phenylalanine (P)        |  | $8.9 \pm 0.5$                           | $11.6 \pm 0.6$ (30.0%)*                 |
| Tryptophan (Trp)         |  | $12.1 \pm 0.4$                          | $17.3 \pm 1.5$ (42.5%)*                 |
| (B)                      |  | Fasting (1000 h)                        | Postprandial (1600 h)                   |
| Ratio                    |  |                                         |                                         |
| Total plasma Trp         |  |                                         |                                         |
| Sum of NAA               |  | $0.2156 \pm 0.0108$                     | $0.2189 \pm 0.0139$ (1%) NS             |
| Free plasma Trp          |  |                                         |                                         |
| Sum of NAA               |  | $0.0086 \pm 0.0012$                     | $0.0043 \pm 0.0002$ (-50%)*             |

Each value represents the average of six subjects  $\pm$  s.e.m.

%, Percentage change between fasting and postprandial samples.

Sum of NAA equals sum of V + I + L + T + P in  $\mu\text{g ml}^{-1}$ .

Statistical significance: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; NS, not significant.

time; at 1200 h fourteen ate a random balanced diet, hospital lunch (diet *a*, 25 g protein, 25 g fats, 55 g carbohydrates, totalling 545 calories); six received equivalent calories in the form of 60 g roast turkey, 100 g steamed rice, 20 g bread, 10 g butter, 100 g unsweetened apple sauce and 240 ml of whole milk (diet *b*) to control for the randomness of the diet. As Table 1 shows all subjects who received diet *a* and diet *b* showed statistically significant changes between the fasting (1000 h) and postprandial (1600 h) samples: postprandial levels of total and bound plasma Trp increased whereas free plasma Trp levels were consistently decreased. Postprandial levels of CSF-Trp and CSF-5-HIAA were also decreased in the 1600 h samples. Significant negative correlations between total or bound plasma Trp and CSF-Trp or CSF-HIAA were obtained regardless of diet and it is unlikely that these changes are due to a diet artefact. Positive non-significant correlations between free plasma Trp and CSF-Trp or CSF-5-HIAA were obtained. If Table 1 is interpreted without considering the food intake status of the subjects, a negative correlation between total plasma Trp and CSF-Trp or CSF-5-HIAA is still obtained during the two samplings, but the correlations between free plasma Trp and CSF-Trp and CSF-5-HIAA were only positive during the fasting (1000 h) samplings. The percentage changes during the postprandial period in total and bound plasma Trp and CSF-HIAA were greater with diet *b*, whereas they were greater for free plasma Trp with diet *a*. In nine subjects who fasted throughout the test, no significant postprandial changes in CSF-5-HIAA were observed between the two samples (difference:  $0.70 \pm 3.5$  ng/ml $^{-1}$ ,  $P > 0.05$ ). CSF-Trp or total plasma Trp was not measured in these subjects, but it is also unlikely that the postprandial changes in total, bound, and free plasma Trp and CSF-Trp are due to diurnal variations<sup>4,7</sup>.

In subjects eating diet *b*, plasma levels of valine, isoleucine, leucine, tyrosine and phenylalanine increased significantly as illustrated (Table 2A). The greatest postprandial increments in plasma NAA were observed in isoleucine and leucine (61.6% and 59.8%, respectively), and the smallest in valine and phenylalanine fractions (32.7% and 30.0%, respectively). As Table 2B shows, the postprandial ratio of total plasma Trp to NAA was not changed, whereas the postprandial ratio of free plasma Trp to NAA was reduced (-49.5%,  $P < 0.01$ ). There was significant positive correlation between the ratio of free Trp to NAA and CSF-Trp or CSF-HIAA, but not between the ratio of total plasma Trp to NAA and CSF values.

Since Trp binds avidly to plasma albumin, an increase in this protein can influence the transport of this amino acid into brain. All subjects showed an increase in serum proteins and plasma albumin after feeding. Serum proteins were in-

creased from  $6.70 \pm 0.37$  g per 100 ml to  $7.05 \pm .40$  g per 100 ml. Serum albumin was increased from  $3.86 \pm 0.018$  g per 100 ml to  $4.04 \pm 0.17$  g per 100 ml. These increments in plasma albumin represent an increase in the availability of binding sites for plasma Trp. Although NEFA were not measured, it is well known that ingestion of balanced or CHO diets decreases plasma NEFA<sup>16</sup>. Measurements of the plasma triglycerides were considered more valuable, because diets rich in CHO produce significant increments in plasma triglycerides which influence the mobilisation of NEFA from fat and liver<sup>23</sup>. All subjects developed a significant postprandial hypertriglycercaemia. Serum triglycerides increased from  $87.66 \pm 10.80$  mg per 100 ml to  $136.00 \pm 18.25$  mg per 100 ml. The increment in dietary and liver triglycerides after feeding is probably responsible for the significant decrease in plasma NEFA, and hence may influence the albumin-binding of Trp by decreasing the extent of saturation of albumin by NEFA.

Determinations of precursors such as Trp, and metabolites of 5-HT such as 5-HIAA, in lumbar CSF have been used widely to estimate the metabolic state of 5-HT in the human central nervous system, where more direct measurements are impossible<sup>24</sup>. There is abundant evidence that changes in lumbar CSF reflect changes in brain<sup>25</sup>. It is now certain that lumbar CSF-5-HIAA does not all derive from the lumbar cord as reported by Bulat *et al.*<sup>26</sup>, and using their experimental conditions it has been shown that 40% to 50% derives from brain 5-HIAA<sup>27</sup>. It has also been estimated that in humans, 23% to 37% of the lumbar CSF-5-HIAA is derived from spinal 5-HIAA<sup>28</sup>. The decrease of CSF-Trp and CSF-5-HIAA after feeding suggests that similar changes occur in the human brain. These facts suggest that the synthesis of brain 5-HT is decreased after ingestion of food in humans. In rodents balanced diets produce a decrease in the synthesis of 5-HT<sup>7</sup>. Our values of CSF-Trp and CSF-5-HIAA are within the range of values reported by others for human CSF<sup>24</sup>.

There is general agreement that CHO or balanced diets increase plasma Trp (total and bound) and decrease free Trp and NEFA in the plasma<sup>3,10</sup>. It is obvious that an increment in NAA is associated with a decrement in brain Trp, and that perhaps the ratios of total plasma Trp to NAA or free plasma Trp to NAA more accurately reflect the changes in brain Trp. In CHO diets we have found that although the levels of free plasma Trp were decreased, the ratio of free plasma Trp to NAA and the ratio of total plasma Trp to NAA were significantly increased ( $P < 0.01$ , manuscript in preparation). In humans, the ratio of total plasma Trp to NAA did not reflect changes in CSF-Trp or CSF-5-HIAA, whereas the ratio of free plasma Trp to NAA



was significantly correlated with changes in CSF values. This indicates that the ratio of free plasma Trp to NAA is the most important factor controlling the transport of plasma Trp into brain, and that this ratio reflects changes in brain Trp not reflected by either total plasma or free plasma Trp. This hypothesis does explain why CHO diets should increase brain Trp while routine diets should decrease it. In CHO diets free Trp to NAA is increased and in balanced diets the free Trp to NAA is decreased. Further experimentation will be necessary to establish definitively the generality of this new hypothesis.

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Received December 6, 1972; final revision January 14, 1974.

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have now been able to study the distribution of the enzyme in specific nuclei of the rat brain. This has been made possible by the development of a technique for the dissection of small areas and individual nuclei<sup>8</sup> of the brain, and the availability of a sensitive assay for the measurement of PNMT. Our findings suggest that adrenaline may serve as a neurotransmitter in the central nervous system.

Sprague-Dawley male rats (200–250 g) were killed by decapitation, and their brains were removed immediately and frozen on dry ice. Frontal serial sections 300  $\mu$ m thick were cut from the border between the spinal cord and the medulla oblongata, at the level of the pyramidal decussation, as far rostral as the level of the anterior commissure. Regions rich in catecholamine-containing cell bodies were removed under stereomicroscopic control<sup>8</sup> as follows. The A1 region<sup>9</sup> (equivalent to the C1 region of Hökfelt *et al.*<sup>10</sup>), was located in the lateral reticular nucleus of the medulla oblongata, between the pyramidal tract and the tractus spinalis nucleus trigemini just under the ventral surface of the medulla oblongata. (Details of the various dissections mentioned here will be published elsewhere.) The A2 region (probably the same as the C2 region of Hökfelt *et al.*<sup>10</sup>) contains catecholamine cell bodies at the level of the region of the vagal nuclei (nucleus commissuralis, nucleus originis dorsalis vagi, nucleus tractus solitarii), and was removed from an area just dorsolateral to the central canal. The A5 region contains catecholaminergic cell bodies, most of which lie between the superior olive and the facial nerve, close to the ventral surface of the brain. The A6 region (locus coeruleus), was removed from the tegmentum medial to the superior cerebellar peduncle. The A7–A8 cell bodies (part of the nucleus cuneiformis) are found at the level of the border of the mesencephalon and pons, dorsal to the medial lemniscus and medial to the lateral lemniscus.

Cortical, limbic and hypothalamic regions were removed with a microdissecting knife. Samples from the septum, preoptic area and caudate-putamen were removed from sections at the anterior commissure level, where the septum is delimited by the lateral ventricles, the corpus callosum and the anterior commissure. The preoptic region lies between the anterior commissure and the ventral surface of the brain medial to imaginary vertical lines drawn through the lateral ventricles.

Samples of tissue from the medial basal hypothalamus (including median eminence, arcuate nucleus and a part of the ventromedial nuclei) were dissected from sections at the median eminence level. The amygdala was removed from the same section, and was limited by the optic tract medially, the caudate-putamen dorsally and the external capsule-piriform cortex laterally. Samples were dissected from the cortex and hippocampus.

The hypothalamic nuclei, the olfactory tubercle and the nucleus interstitialis striae terminalis, were removed, and both the compact and reticular zones of the substantia nigra were dissected between the lemniscus medialis and the crux cerebris.

PNMT in brain samples was measured by a modification of the method previously described<sup>2</sup> as follows. Brain tissue from one rat was homogenised in 65  $\mu$ l of 0.2 M Tris-HCl buffer, pH 8.6, and 5  $\mu$ l was removed for protein determination<sup>11</sup>. After centrifugation, 50  $\mu$ l of the supernatant was transferred to 13-ml glass-stoppered centrifuge tubes. The reaction was initiated by addition of 100  $\mu$ l of a solution containing 4.5  $\mu$ mol of phenylethanolamine and 0.54 nmol of <sup>3</sup>H-methyl-S-adenosyl-1-methionine (New England Nuclear Corp., specific activity 4.5 mCi  $\mu$ mol<sup>-1</sup>). Blanks were prepared by omitting the substrate from the incubation mixture. The tubes were incubated for 30 min at 37° C. The reaction was stopped by the addition of 0.5 M borate buffer, pH 10, and the radioactive product was separated from the <sup>3</sup>H-methyl-S-adenosyl-1-methionine by extraction into toluene

## Localisation of phenylethanolamine N-methyl transferase in the rat brain nuclei

THE conversion of noradrenaline to adrenaline is catalysed by phenylethanolamine N-methyl transferase (PNMT, EC 2.11.1.2), which is highly localised in the adrenal medulla. Small amounts of PNMT-like enzyme<sup>2–5</sup> and adrenaline<sup>6,7</sup> have been detected in the central nervous system, and we

TABLE 1 PNMT activity in rat brain nuclei

| Region                                                    | PNMT           |
|-----------------------------------------------------------|----------------|
| A1 (C1)                                                   | 41.5 $\pm$ 5   |
| A2 (C2)                                                   | 40.3 $\pm$ 4.3 |
| A5                                                        | 5.6 $\pm$ 1.4  |
| A6 (locus coeruleus)                                      | 12.0 $\pm$ 1.2 |
| A7-A8                                                     | 6.1 $\pm$ 1.1  |
| Cerebellar cortex                                         | Not detectable |
| Frontal cortex                                            | Not detectable |
| Cingulate cortex                                          | Not detectable |
| Piriform cortex                                           | Not detectable |
| Hippocampus                                               | Not detectable |
| Amygdala                                                  | Not detectable |
| Septum                                                    | 6.3 $\pm$ 1.7  |
| Nucleus interstitialis striae terminalis                  | 6.7 $\pm$ 0.8  |
| Olfactory tubercle                                        | Not detectable |
| Preoptic region                                           | 7.7 $\pm$ 0.7  |
| Hypothalamus                                              |                |
| Basal hypothalamus                                        | 15.9 $\pm$ 2.2 |
| Nucleus periventricularis                                 | 8.0 $\pm$ 0.9  |
| Medial forebrain bundle (anterior)                        | 6.2 $\pm$ 0.5  |
| Nucleus dorsomedialis                                     | 10.9 $\pm$ 3.2 |
| Nucleus supraclasmatis                                    | 5.6 $\pm$ 0.7  |
| Nucleus supraopticus                                      | 5.2 $\pm$ 1.3  |
| Nucleus anterior                                          | 5.9 $\pm$ 1.3  |
| Nucleus paraventricularis                                 | 15.0 $\pm$ 2.5 |
| Area retrochiasmatica                                     | 9.7 $\pm$ 3.4  |
| Median eminence                                           | 15.6 $\pm$ 3.0 |
| Nucleus arcuatus                                          | 10.3 $\pm$ 2.0 |
| Nucleus ventromedialis                                    | 8.3 $\pm$ 1.6  |
| Nucleus perifornicalis                                    | 8.1 $\pm$ 1.6  |
| Nucleus premammillaris dorsalis                           | 4.1 $\pm$ 0.5  |
| Nucleus premammillaris ventralis                          | 4.9 $\pm$ 1.4  |
| Nucleus posterior hypothalami                             | 5.1 $\pm$ 1.0  |
| Medial forebrain bundle<br>(posterior hypothalamic level) | 5.3 $\pm$ 1.2  |

Brain samples were dissected and processed as described in the text. Results represent mean  $\pm$  s.e.m. for groups of eight animals. One unit of enzyme activity is equal to 1 pmol of <sup>3</sup>H-N-methyl phenylethanolamine formed per mg of protein and per hour of incubation.

containing 3% of isoamyl alcohol (v/v). A 5 ml sample of the organic solvent was evaporated to dryness under a stream of air, and the radioactivity remaining was counted. The whole procedure was repeated using internal standards consisting of 1 U of purified PNMT<sup>13</sup>, and the results were corrected accordingly. The use of a methyl donor of high specific activity and a drying procedure to eliminate a volatile radioactive contaminant increased the sensitivity of the assay fifty-fold over that previously in use<sup>13</sup>.

No enzymatic activity was found when phenylethanolamine was replaced by  $\beta$ -phenylethylamine or tryptamine as substrates for the reaction. This finding suggests that the enzyme studied is PNMT, rather than the non-specific N-methyl transferase described earlier<sup>12</sup>.

Our results indicate that PNMT activity is unevenly distributed in the rat brain, and is localised in specific areas. The greatest PNMT activity was measured in two areas, A1 and A2, which are rich in catecholamine-containing cell bodies<sup>9</sup>. Other areas containing many catecholaminergic neurones, such as A5 and A7-A8, were low in PNMT activity, and the region of the locus coeruleus (A6) showed intermediate values (Table 1). These results indicate that PNMT is not distributed homogeneously among regions rich in catecholamine-containing neurones.

PNMT activity could not be detected in any of the cortical areas examined, in the caudate, the amygdala or the olfactory tubercle (Table 1). The substantia nigra, the nucleus interstitialis striae terminalis and the septum showed relatively low levels of activity.

Enzymatic activity was detected in all of the hypothalamic nuclei studied as well as in the preoptic region. The PNMT activity was found to be lower than the dopamine- $\beta$ -hydroxy-

lase and tyrosine hydroxylase activities found in similar hypothalamic nuclei.

PNMT was found to be unevenly distributed within hypothalamic nuclei. The largest concentrations were in the medial basal hypothalamus in the arcuate and paraventricular nuclei and in the median eminence. Intermediate concentrations were found in the paraventricular, dorsomedial, ventromedial and perifornical nuclei and in the area retrochiasmatica. Small concentrations were found in the rest of the hypothalamic nuclei.

Adrenaline and PNMT are present in high concentrations only in the adrenal medulla<sup>1,2</sup>. PNMT and its product seem also to be present in nerves, however. Work with amphibians has provided evidence that PNMT is synthesised and transported in the axons of peripheral nerves<sup>14</sup>. Small quantities of PNMT<sup>2-5</sup> and adrenaline<sup>6,7</sup> have been measured in the brain of mammals. Hökfelt *et al.*<sup>10</sup> reported an immunohistological technique for the visualisation of PNMT in the brain. The localisation of the enzyme that we report agrees well with the histological data.

The medial basal hypothalamus, a region rich in the catecholamines dopamine and noradrenaline (M.P. *et al.*, submitted for publication), plays an important role in neuroendocrine regulation. The fact that PNMT is concentrated in this region suggests that adrenaline can also be produced there. Thus adrenaline may have a role in the hypothalamic control of the anterior pituitary.

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## Learning impairment in rats after 6-hydroxydopamine-induced depletion of brain catecholamines

STEIN<sup>1</sup> suggested, on the basis of the effects of amphetamine and other adrenergic drugs on self-stimulation behaviour, that the reinforcement feedback of the consequence of an action was mediated by a noradrenergic pathway in the region of the medial forebrain bundle. Noradrenergic and dopaminergic pathways in this region of the brain have been mapped by Ungerstedt<sup>2</sup>, and Crow<sup>3,4</sup> has suggested that the gustatory and olfactory input to these pathways might fit them for a role in transmitting environmental feedback. Other evidence implicates the dopaminergic nigro-striatal pathway in memory function as well. Microstimulation in the pars compacta of the substantia nigra has been found to produce a memory impairment not found on stimulation of the surrounding nuclei<sup>5</sup>, lesions in the substantia nigra impair learning<sup>6,7</sup> and pharmacological manipulation of the level of dopamine in the substantia nigra can impair learning and performance of rats in a delayed response task<sup>8</sup>. Crow<sup>9</sup> has found that locus coeruleus lesions may completely abolish learning in a runway situation. This indicates the involvement of monoaminergic-containing neurones in memory function. 6-Hydroxydopamine produces a depletion of brain noradrenaline and dopamine when injected into the lateral ventricle of the rat brain<sup>10</sup>, and also a reduction in the activity of the biosynthetic enzyme tyrosine hydroxylase<sup>11</sup> consistent with the morphological finding of acute degeneration of the nerve terminals<sup>12,13</sup>. Depletion of the monoaminergic neurones using 6-hydroxydopamine would therefore be predicted to impair learning behaviour, and has indeed been found to produce a transitory decrease in responding on a fixed-ratio schedule for water reward<sup>14</sup>. This, however, returned to normal within a few days. Conversely, an increase in responding on a variable interval schedule after 6-hydroxydopamine has been found<sup>15</sup>.

This latter effect, however, was shown to be on the emission of responses after training to a stable level and not on the learning process itself. Our results using a demanding motor-learning task, show severe impairment after 6-hydroxydopamine during acquisition itself, which is not due to gross motor paralysis or hypokinesia.

Twenty experimentally naive male albino Wistar rats, weighing about 200 g at the time of operation, were anaesthetised with ether and two doses of 6-hydroxydopamine, (150 µg dissolved in 15 µl of 0.9% saline with 1 mg ml<sup>-1</sup> ascorbic acid antioxidant) were injected, one each side, into the lateral ventricle 2 mm lateral and 1–2 mm posterior to the bregma in ten animals. Controls received injections of the vehicle solution. Both groups were pretreated with the monoamine oxidase inhibitor tranylepromine (5 mg kg<sup>-1</sup>) 30 min before the operation to enhance the depletion of dopamine<sup>16</sup>.

Two treated animals died during the course of the experiment. Pretraining was begun approximately 10 weeks after treatment, acquisition started 2 weeks later and transfer 4 weeks after this. The animals were trained on a complex task in which they had either to pull or to push a ball out of a perspex tunnel to reach food<sup>17</sup>. The apparatus comprised two open-topped boxes 25 cm high, and base 22 cm × 25 cm, connected at floor level by a 23 cm long cylindrical plexiglass alley of inside diameter 10 cm. The boxes were identical except that one, the goal box, contained a food dish into which sugar pellets could be delivered by a Gerbrands feeder. The alley could be partially obstructed by a plastic ball, 8 cm in diameter and weighing 45 g, placed equidistant from the start and goal boxes. Attached to the ball by a rubber suction cup was a 3.8 cm handle made from a 4 BA screw. Movement of the ball in either direction could be blocked by metal pegs mounted on pivots over the top of the alley and protruding down from the roof through a longitudinal slit in the plexiglass. Both pegs were always left hanging down, although only one was locked so that the rat had no obvious clue as to which of the two responses was possible on any trial. The apparatus was weakly illuminated and the rat was observed by mirrors placed at 45° above the apparatus. The subjects were placed on a 2 h d<sup>-1</sup> food access schedule until their weights were approximately 85% of free feeding weight. Subsequently they were fed 15 g d<sup>-1</sup> of laboratory chow, immediately after the subject's last trial of the day. On day 1 of pretraining, the subjects were placed in the start box and given 5 min exposure to the apparatus with the ball removed, pegs hanging down and food cup overflowing with sugar pellets. Fifteen further trials were given in which now only 2 pellets were available in the food cup per trial. A further 34 trials were given in which the pellets were delivered by the Gerbrands feeder, one on entry to the goal box and another 4 s later. Acquisition testing was now started with the ball placed in the tunnel and the distal peg locked so that the ball could only be pulled out of the tunnel. The subject was given a 5 min trial to solve the problem and then removed from the apparatus if the ball was still in place. If the ball had been moved part of the way out by the end of 5 min a further 5 min was allowed, but the subject unconditionally removed at the end of this time. The amount of time spent actually in contact with the ball was noted, this being taken as a measure of how much the rat was trying to move the ball ('trying time') and hence being a better measure than 'total-time-in-the-apparatus-to-solution' which is made up of behaviour other than that oriented towards the ball. The movements of head and paws used in manipulating the ball were also noted. Forty-seven trials were given in all, and the cumulative 'total-time-spent-in-the-apparatus-before-solution' of the task noted for each animal. By trial 28, one treated and two controls had failed to solve the task and showed no sign of doing so. 'Shaping trials' in which the ball was placed half in and half out of the tunnel and then moved further back were given. These animals were classified as

TABLE 1 Performance on ball-in-tunnel task.

|                           | Acquisition            |                     | Transfer one               |                            | Transfer two                 |                              |
|---------------------------|------------------------|---------------------|----------------------------|----------------------------|------------------------------|------------------------------|
|                           | Time to solution (min) | Trying time (s)     | Time to solution (min)     | Trying time (s)            | Time to solution (min)       | Trying time (s)              |
| 6-hydroxydopamine (n = 8) | 65.8 ± 14.7            | 572 ± 126           | 45 ± 15                    | 29 ± 12                    | 6.8 ± 2.4                    | 4.3 ± 0.9                    |
| Controls (n = 8)          | 8.75 ± 1.9<br>P, 0.05  | 80 ± 14<br>P, 0.001 | 87 ± 26<br>Not significant | 49 ± 18<br>Not significant | 7.0 ± 0.5<br>Not significant | 4.9 ± 0.5<br>Not significant |

Performance of controls and 6-hydroxydopamine treated animals on acquisition, transfer 1 and transfer 2 of the ball-in-tunnel task. 'Time to solution' is the total time spent in the apparatus before solving the task; 'trying time' is the time spent in contact with the ball manipulating it. Values given are means and standard deviations for each group.

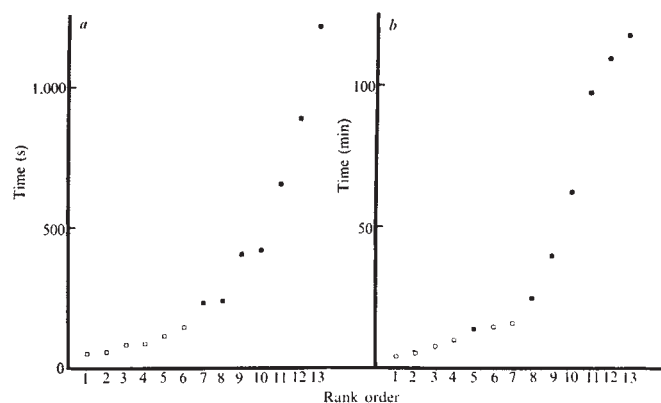


FIG. 1 Trying time (a) and time in apparatus (b) for the control and treated animals plotted against their position in the overall rank order of times for both groups. The animals separate out into two distinct groups, that is rapid solution controls and slow solution treated, with very little overlap. Of the 16 subjects used, two controls and one treated failed to solve acquisition within 28 trials and were dropped from the analysis of acquisition times. □, Controls; ■, treated.

failures and dropped from the analysis of acquisition. At the end of 47 acquisition trials, the proximal peg was locked and the other released and transfer testing begun with the animals being transferred from pull to push during the course of 28 trials. A second transfer phase was then started in which the animals were transferred back to pull. The total time in the apparatus for successful transfer was noted in each case. (Table 1).

On both 'trying time' and 'total-time-in-apparatus-to-solution' the 6-hydroxydopamine animals were severely impaired relative to the controls. (Fig. 1). On both transfer 1 and transfer 2, there was no significant difference between the groups, all animals mastering the second transfer within one 5 min trial. At the end of transfer 2 the animals were killed by decapitation and the brains rapidly removed, chilled and dissected into regions according to the method of Glowinski and Iversen<sup>18</sup>. The striatum, hypothalamus and cortex were homogenised in distilled water (1:10(w/v)) and 10  $\mu$ l aliquots taken for radiochemical assay of tyrosine hydroxylase<sup>19,20</sup>, the results of which confirmed that the 6-hydroxydopamine animals had sustained severe losses of adrenergic neurones in the hypothalamus, cerebral cortex and corpus striatum (Table 2).

The severe impairment in acquisition in the treated animals cannot be attributed to a motor impairment. The general mobility and time spent in the tunnel was the same in both groups. Each treated animal showed the normal range of head and paw movements and the frequency of these movements was the same in both groups. It also seems unlikely that the deficit is motivational as the treated animals spent as much time as controls in behaviour orientated towards the ball (measured as cumulative time in physical contact with the ball for each trial). Although this measure decreased after the first few trials as the animals habituated to the ball, it decreased equally for both groups, from an initial value that was virtually identical for both treated and control groups.

TABLE 2 Tyrosine hydroxylase activity (DOPA formed pmole  $h^{-1} g^{-1}$  wet weight).

|                   | Striatum        | Hypothalamus   | Cortex          |
|-------------------|-----------------|----------------|-----------------|
| 6-Hydroxydopamine | 35.6 $\pm$ 12.0 | 19.4 $\pm$ 4.5 | 2.48 $\pm$ 2.32 |
| (n = 8)           | (8.1%)          | (16.4%)        | (14.9%)         |
| CONTROLS          | 438 $\pm$ 25.5  | 118 $\pm$ 8.7  | 16.7 $\pm$ 2.4  |
| (n = 8)           |                 |                |                 |

Mean and standard error of the tyrosine hydroxylase activity of the three brain areas (see text). These values in the 6-hydroxydopamine-treated animals are also expressed as % control value for that region.

Nor do the treated animals suffer from a retention defect, as once they had mastered the task they, without exception, performed perfectly on every subsequent trial and did not show the pattern of occasional failures expected with a retention defect. Thus 6-hydroxydopamine treatment produced a severe impairment in the actual learning process itself, as distinct from retention or emission of responses. Two related questions remain for future research. First, is the deficit specific to learning the complex motor sequences required for this demanding task, or general to all learning dependent on reinforcement? Second, dopaminergic systems are implicated in complex motor functions and noradrenergic systems with reinforcement mechanisms. The intraventricular 6-hydroxydopamine treatment severely depletes both dopamine and noradrenaline from the forebrain and selective lesion techniques using 6-hydroxydopamine will be employed to determine whether one amine rather than the other underlies the learning deficit.

We thank Dr L. L. Iversen for his help in performing the tyrosine hydroxylase assays, C. D. Andrews for performing the 6-hydroxydopamine injections, and Dr M. J. Morgan for allowing the use of his apparatus.

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Received November 26, 1973; revised January 30, 1974.

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## Activation of human T and B lymphocytes by polyclonal mitogens

LYMPHOCYTES can be activated *in vitro* by a variety of so-called polyclonal stimulants or mitogens (reviewed in refs 1, 2 and 3). These responses characteristically involve a larger proportion of cells than observed with antigen as the eliciting ligand, and the initiating events are widely believed to be independent of any immunogenicity possessed by the stimulant. These features of polyclonal responses provide the rationale for the use of such systems both as a model for the study of activation processes in lymphocytes<sup>2</sup>



TABLE 1 Cell surface markers and cell populations used

|                         | T lymphocyte markers* |            | B lymphocyte markers* |            | EAC (C3)¶  |
|-------------------------|-----------------------|------------|-----------------------|------------|------------|
|                         | anti-T sera†          | SRFC§ (E)  | anti-B sera‡          | anti-Ig    |            |
| 1 Tonsil**              |                       |            |                       |            |            |
| Unseparated             | 52.8 ± 1.9            | 54.8 ± 1.6 | 44.5 ± 2.3            | 43.2 ± 1.8 | 34.0 ± 2.3 |
| T cells: single cycle†† | 95(4)                 | 94 ± 0.8   | 1.8 (4)               | 2.1 ± 0.3  | 0.2(2)     |
| B cells: single cycle   | 1.3(3)                | 1.9 ± 0.3  | 97.1(4)               | 96.7 ± 1.5 | 86.4(3)    |
| double cycle‡           | —                     | <0.06      | —                     | 96.8       | —          |
| 2 Spleen**              |                       |            |                       |            |            |
| Unseparated             | —                     | 32.2(4)    | —                     | —          | —          |
| B cells: double cycle‡  | —                     | <0.05      | —                     | —          | —          |

\* Cell surface markers for human T and B lymphocytes reviewed in refs 17 and 18. Detailed methods for these markers are published elsewhere<sup>19,29,30</sup>. Values given represent mean ± standard error of five to twenty-seven determinations. Mean value only given where number of observations (in parentheses) was less than five—means not tested.

† Rabbit anti-human brain serum absorbed with chronic lymphocytic leukaemia (CLL) cells.

‡ Rabbit anti-CLL cell serum absorbed with thymocytes<sup>30</sup>.

§ Spontaneous rosette formation at room temperature with sheep erythrocytes (<2-week-old).

|| Rabbit anti-human immunoglobulin (polyspecific).

¶ Rosette-forming cells with receptor for C3. Sheep red cells + rat IgM anti-sheep red cell antibody + mouse serum (complement). Test performed on a rotatory shaker at 37°C.

\*\* Tonsils were obtained from patients undergoing tonsillectomy for recurrent throat infections. Spleens used in these experiments were obtained from accident cases. Single cell suspensions prepared using an Ultra-Turrax homogeniser followed by filtration directly through glass wool at room temperature to remove aggregates of cells.

†† T cells purified by filtration of T and B cells through columns of nylon fibres (Fenwall Leukopak)<sup>19,31,32</sup>. Optimal conditions are described elsewhere<sup>19</sup>. Yields of T cells were 49 ± 2.9 (s.e.)% .T cell suspensions contained less than 1% cells capable of phagocytosing polystyrene particles in 50% foetal calf serum at 37°C.

‡‡ B cells were purified by removing those (T) cells forming spontaneous (E) rosettes by density centrifugation in Ficoll-isopaque<sup>19</sup>. Re-rosetting and a second separation (double cycle) was necessary to reduce T cell contamination to less than 0.1%. B cell yields were 62–83%. The proportions of phagocytic cells was not routinely assessed in these populations but is likely to be somewhat enriched at least with respect to monocytes, compared with the initial cell suspensions. Tonsil cell suspension contained 0.5% to 2% phagocytic cells and spleen cells 8% on the single occasion that they were assayed. Blood monocytes purified by the method of Huber and Fudenberg<sup>33</sup> were unreactive with all antisera used and did not form spontaneous rosettes. In a single experiment they did not form rosettes with EAC (mouse).

and clinically for the assessment of proliferative potential of blood-borne lymphocytes<sup>4</sup>.

Both of these aims have been facilitated by the finding that T and B lymphocytes in mice respond selectively to all mitogens so far tested with the exception of pokeweed mitogen (PWM), which stimulates both T and B cells<sup>5,6</sup> and may in fact contain separate T and B mitogens. (F. Loo, personal communication). This selectivity is most clearly observed with lectins (such as phytohaemagglutinin (PHA) and concanavalin A (conA) and bacterial lipopolysaccharides which activate T and B cells respectively<sup>1-3</sup>. Most polyclonal mitogens do however seem to bind equally well to both 'responder' and 'non-responder' cell types<sup>7-9</sup>, and under appropriate circumstances (for example, insolubilisation of lectins<sup>10-11</sup>) responses can be induced in a normally unresponsive population.

These convenient relationships are challenged by recent observations; the first, that some mouse<sup>1,12</sup> and human<sup>13</sup> B lymphocytes do respond to T mitogens in the presence of activated T cells, and second that human tonsillar B lymphocytes purified by a digestible immunoabsorbent method respond directly to PHA<sup>13,14</sup>. The latter observation contradicts earlier circumstantial but nevertheless compelling evidence from studies on patients with selective immunodeficiency diseases which suggested that PHA was selective for T cells in man<sup>15</sup>. Indeed on the basis of these results and other data from mice, rats and chickens<sup>16</sup> the proliferative response to PHA has been widely used to assess T cell status in man<sup>4</sup>.

We have investigated this problem by the application of a series of cell surface markers<sup>17,18</sup> which readily distinguish T and B cells in man (Table 1). The T or B cell origin of activated lymphoblasts has been assessed in cultures containing T plus B cells, B cells only or T cells only. Purification of T and B lymphocytes was carried out by negative selection: T cells were eliminated by density sedimentation of cells which had formed 'spontaneous' rosettes with sheep erythrocytes and B cells removed by adherence to nylon fibres<sup>19</sup>. Lymphocytes from tonsils, spleen and blood have been extensively studied with different culture conditions, media and mitogen concentrations.

Figure 1 illustrates representative results using optimal doses of PHA and PWM to stimulate tonsil and spleen lymphocytes. Tonsillar T cells responded well to both PHA and PWM. B lymphocytes were not activated by PHA and gave only a small response with PWM. In cultures containing both T and B cells, T lymphoblasts predominated in the response to both PHA and PWM. But up to 15% of the PHA-activated lymphoblasts in tonsil cells cultures with either Dulbecco's or RPMI medium on day 3 may be B cells. Repeat experiments on tonsils and also with blood lymphocytes have given consistent results. The response profile of spleen cells in RPMI medium was different from that of tonsils. T lymphoblasts again predominated the PHA response of unseparated cells (85%); however the majority (88%) of lymphoblasts in PWM stimulated cultures were B cells. Purified B lymphocytes responded well to PWM but as with tonsil B cells no activation was detectable with PHA. Weak but significant spleen B lymphocyte responses have also been induced by *Escherichia coli* lipopolysaccharide (LPS) and staphylococcal enterotoxin B. (unpublished observations of G. J. and M. F. G.).

These results with human lymphocytes parallel our earlier observations of the cell types responding to PHA and PWM in mice<sup>2</sup>, but they contradict those of Phillips *et al.*<sup>13,14</sup>. While it can be agreed that the response to PHA of mixed T plus B lymphocyte cultures may involve a small and perhaps variable B cell component we could not confirm the results suggesting that purified B cells respond to PHA independently of T cells. The failure of purified human B lymphocytes to respond to PHA has also recently been found when cell purification was achieved by using cytotoxic anti-T lymphocyte sera (refs 20 and 21, and M. Cooper, personal communication). In addition there is a considerable body of clinical data which illustrates clearly that lymphocytes from patients with thymic aplasia which are almost entirely B cells do not respond to PHA<sup>22,23</sup>. Responsiveness can however be instated by T cell reconstitution following thymus grafting<sup>22</sup>.

Despite this compelling weight of evidence the positive result of Phillips and Roitt cannot be ignored and demands an explanation. If we assume that the discrepancies do not

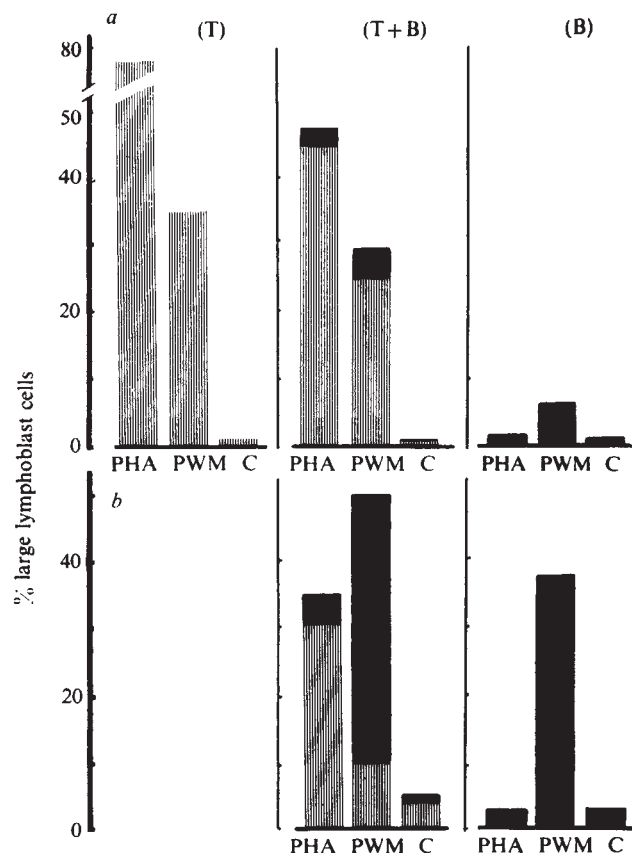


FIG. 1 Response of human T and B lymphocytes to phytohaemagglutinin and pokeweed mitogen *in vitro*. □, Lymphoblasts positive for T cell surface markers. ■, Lymphoblasts positive for B cell surface markers. a, Tonsil; b, spleen. The culture methodology was essentially as described previously<sup>16,28</sup>. Here tonsil cells were cultured in Dulbecco's Modified Eagle's Medium with 10% heat-inactivated human AB serum, and spleen cells in RPMI 1640 with 10% heat inactivated foetal calf serum. PHA was the purified preparation from Burroughs Wellcome and the responses shown are with  $2 \mu\text{g ml}^{-1}$  assayed on day 3. PWM has protein derivative of *Phytolacca americana* prepared by TCA precipitation of a crude saline homogenate of stems and roots. The responses shown are with  $10 \mu\text{g ml}^{-1}$  on day 3. The proportions of T and B cells in the cultures at 0 h were within the range indicated in Table 1. T lymphoblasts were typed as (S-RFC<sup>+</sup>, Ig<sup>+</sup>, B-Ag<sup>+</sup>) and B lymphoblasts as (S-RFC<sup>+</sup>, Ig<sup>+</sup>, B-Ag<sup>+</sup>), see Table 1. In additional experiments presumptive T and B lymphoblasts were also typed with EAC, anti-T cell serum and an anti-T lymphoblast serum analogous to that described by Phillips and Thomas<sup>14</sup>. Results with these markers paralleled very precisely those with the three markers used routinely. Aggregates of lymphoblasts were disrupted by vigorous pipetting and in occasional experiments with an Ultra-Turrax homogeniser. Rosetted suspensions were stained with 0.1% toluidine blue and viewed under ordinary light. Binding of anti-T, anti-B and anti-Ig sera to lymphoblasts in suspension was assayed using fluorescein-labelled goat anti-rabbit immunoglobulin and viewed with a Vickers M41 Photoplan fluorescence microscope with incident illumination. In all assays only cells with diameters greater than  $10 \mu\text{m}$  were scored as lymphoblasts. Analysis of May-Grunwald/Giemsa stained smears confirmed that over 95% of enlarged cells were indeed lymphoblasts. Absolute numbers of lymphoblasts were counted and the response expressed as the number of lymphoblasts in relation to the initial cell number in the culture.

relate to quantitative problems or mistaken cellular identity then the following possibilities seem to us as the only plausible explanations for the discrepancy: our methodology may be inadequate for B cell responses to PHA either because culture conditions themselves are suboptimal and biased towards T cells, or alternatively because we have either lost or suppressed potentially responsive B cells during our purification procedure. We suspect this explanation is

unlikely to be correct since our B cell cultures do respond to PWM by both morphological transformation and DNA synthesis (30% of PWM-stimulated B lymphoblasts incorporate thymidine between days 3 and 4; unpublished observations of G. J. and M. F. G.). Moreover our culture conditions and media do not differ in any obviously significant way from those of Phillips and colleagues (ref. 13 and personal communication from Roitt and Thomas). Clearly however it is not possible at present to entirely rule out this explanation.

Alternatively it may be that the B lymphocyte responses to PHA observed by Phillips *et al.* are dependent upon facilitation mechanisms absent in our and others' systems. Potential factors of significance include the presence of macrophages, small numbers of activated T cells and subliminal stimulation by anti-immunoglobulin antibodies that are released during B cell purification on dextranase digestible Sephadex immunoabsorbent. Evidence can be procured to support all three possibilities. Macrophages have been found to potentiate PHA responses of both T cells<sup>24</sup> and B cell enriched cultures (L. Epstein, W. Kreth and L. Herzenberg, personal communication) and our own results indicate that contamination of B cells with as few as 2% T cells facilitates a PHA response. But this T-cell dependent response is also expressed principally by the 'residual' T cell (unpublished observations). Nevertheless the additional finding that culture supernatants of conA-activated T cells facilitate a small B cell response to this mitogen<sup>25</sup> provides evidence for a T-cell dependent, B cell response. Facilitation of PHA activation by binding of anti-human immunoglobulin antibodies which by themselves may be non- or minimally mitogenic seems to be a tenable explanation since such synergistic effects have been observed between anti-immunoglobulin and antigen in mice<sup>26</sup> and different mitogens in man<sup>27</sup>.

These latter three explanations have at least the virtue that they can be readily tested. Regardless of the eventual outcome of this issue two points emerge which are relevant to the clinical application of proliferative responses to PHA. Under defined conditions the PHA response can be T-cell dependent and expressed predominantly by T lymphocytes themselves. In our hands optimal T cell proliferation with minimal B cell involvement is observed when cells are cultured in Dulbecco's modified Eagle's medium with 10% human AB serum. The PHA response can therefore be used as a quantitative or semiquantitative assay for the proliferative response of blood-borne T cells. A small proportion (5–15%) of B cells may however become activated in T-B cell mixtures (that is, in most clinical situations) and the application of cell surface markers is required to delineate this B cell contribution.

This work was supported by the Imperial Cancer Research Fund and the Medical Research Council of Great Britain. We thank Mr G. Stathopoulos for counting E rosettes in the spleen preparations, Dr J. Sachs of the London Hospital for supplying spleens and colleagues at University College Hospital, London for tonsils (not their own).

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Received December 17, 1973.

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## Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system

RECENT experiments<sup>1-3</sup> indicate that cooperation between thymus derived lymphocytes (T cells) and antibody-forming cell precursors (B cells) is restricted by the H-2 gene complex. Helper activity *in vivo* operates only when T cells and B cells share at least one set of H-2 antigenic specificities. Evidence is presented here that the interaction of cytotoxic T cells with other somatic cells budding<sup>4-5</sup> lymphocytic choriomeningitis (LCM) virus is similarly restricted.

Both the cytotoxic assay used and the characteristics of the cells involved have been described previously<sup>6-8</sup>. Briefly, monolayers of C3H mouse fibroblasts (L cells) are grown in plastic tissue culture trays, infected with a high multiplicity of the WE3 strain of LCM virus and the cells labelled with <sup>51</sup>Cr and overlaid (40:1) with the spleen cell preparation to be tested. Supernatants are removed between 15 and 16 h later and % <sup>51</sup>Cr release calculated<sup>7</sup>. Results are expressed as mean  $\pm$  s.e.m. for four replicates. Cytolysis of

TABLE 1 Cytotoxic activity of spleen cells from various strains of mice injected i.c. 7 d previously with 300 LD<sub>50</sub>\* of WE3 LCM virus for monolayers of LCM-infected or normal C3H (H-2<sup>k</sup>) mouse L cells.

| Experiment | Mouse strain          | H-2 type | % <sup>51</sup> Cr release† |                |
|------------|-----------------------|----------|-----------------------------|----------------|
|            |                       |          | Infected                    | Normal         |
| 1          | CBA/H                 | k        | 65.1 $\pm$ 3.3              | 17.2 $\pm$ 0.7 |
|            | Balb/C                | d        | 17.9 $\pm$ 0.9              | 17.2 $\pm$ 0.6 |
|            | C57Bl                 | b        | 22.7 $\pm$ 1.4              | 19.8 $\pm$ 0.9 |
|            | CBA/H $\times$ C57Bl  | k/b      | 56.1 $\pm$ 0.5              | 16.7 $\pm$ 0.3 |
|            | C57Bl $\times$ Balb/C | b/d      | 24.8 $\pm$ 2.4              | 19.8 $\pm$ 0.9 |
|            | nu/+ or +/+           |          | 42.8 $\pm$ 2.0              | 21.9 $\pm$ 0.7 |
| 2          | nu/nu                 |          | 23.3 $\pm$ 0.6              | 20.0 $\pm$ 1.4 |
|            | CBA/H                 | k        | 85.5 $\pm$ 3.1              | 20.9 $\pm$ 1.2 |
|            | AKR                   | k        | 71.2 $\pm$ 1.6              | 18.6 $\pm$ 1.2 |
| 3          | DBA/2                 | d        | 24.5 $\pm$ 1.2              | 21.7 $\pm$ 1.7 |
|            | CBA/H                 | k        | 77.9 $\pm$ 2.7              | 25.7 $\pm$ 1.3 |
|            | C3H/HeJ               | k        | 77.8 $\pm$ 0.8              | 24.5 $\pm$ 1.5 |

\* Other mice were injected with  $2 \times 10^6$  LD<sub>50</sub>, but levels of specific release were invariably lower due to the high dose immune paralysis<sup>8,20</sup> associated with viscerotropic (WE3) LCM virus.

† % <sup>51</sup>Cr release by normal spleen cells on infected targets ranged from: (experiment 1) 17.1  $\pm$  0.3 to 20.0  $\pm$  0.7; (experiment 2) 20.0  $\pm$  1.4 to 25.3  $\pm$  0.7; (experiment 3) 27.2  $\pm$  2.0.

infected L cells by CBA/H immune spleen cells has been shown to be a property of specifically sensitised thymus-derived lymphocytes, which act in the absence of both macrophages and substances secreted into the medium at large<sup>6-8</sup>.

Various strains of mice were injected intracerebrally (i.c.) with 300 mouse LD<sub>50</sub> of WE3 LCM virus. Mice were sampled 7 d later when, in CBA/H mice, maximal cytotoxic activity is found in lymphoid tissue<sup>6,7</sup>. Only spleen preparations<sup>6</sup> from mice sharing at least one set of H-2 antigenic specificities with the target monolayer caused high levels (40% to 50%) of specific lysis (Table 1). Spleen cells from nude control (nu/+ or +/+) mice, derived locally from CBA and Balb/C stock (Dr J. B. Smith, personal communication), were less active and lymphocytes from histoincompatible mice caused minimal specific release (< 5%) of <sup>51</sup>Cr.

Spleen preparations from mice immunised 10 and 13 d previously were also assayed, as Marker and Volkert<sup>9</sup> have reported that maximal cytotoxicity of C3H lymphocytes for L cells infected with the Traub strain of LCM virus occurs at 11 d after inoculation. High levels of specific <sup>51</sup>Cr release were again recognised only in the histocompatible system (Table 2) activity declining, as has been shown previously<sup>7</sup>, from a peak on day 7.

Demonstration of reciprocal exclusion of cytolysis was essential to establish that mice possessing other than H-2<sup>k</sup> antigenic specificities are capable of generating cytotoxic T cells. Comparisons were thus made using similar targets from allogeneic mouse strains. Peritoneal macrophages were obtained<sup>10</sup> from normal Balb/C and CBA/H mice, cultured in plastic tissue culture trays and infected<sup>11</sup> with WE3 LCM virus. Specific lysis was restricted to the syngeneic system

TABLE 2 % <sup>51</sup>Cr release\* from infected C3H L cells overlaid with spleen cells from mice sampled at 7, 10 and 13 d after intravenous inoculation with 2,000 LD<sub>50</sub> of WE3 LCM virus.

| Mouse strain | Days after inoculation |                |                |
|--------------|------------------------|----------------|----------------|
|              | 7                      | 10             | 13             |
| CBA/H        | 72.0 $\pm$ 2.0         | 66.4 $\pm$ 1.4 | 27.5 $\pm$ 0.5 |
| Balb/C       | 26.1 $\pm$ 0.7         | 28.0 $\pm$ 1.6 | 22.7 $\pm$ 1.8 |
| C57Bl        | 27.3 $\pm$ 1.1         | 24.3 $\pm$ 1.8 | 24.0 $\pm$ 0.4 |

\* Levels of <sup>51</sup>Cr release due to overlaying normal L cells with immune spleen cells, infected L cells with control spleen cells or with medium alone ranged from 17.1  $\pm$  0.4 to 24.0  $\pm$  1.4. Other mice were injected with  $2 \times 10^6$  LD<sub>50</sub>, but levels of specific release were invariably lower.

TABLE 3 %  $^{51}\text{Cr}$  release from normal and infected peritoneal macrophages by spleen cells from control mice and from mice injected i.e. with 300 LD<sub>50</sub> of WE3 LCM virus 7 d previously.

| Spleen cells |                  | Macrophage source | % $^{51}\text{Cr}$ release from macrophages |                             |                             |                             |
|--------------|------------------|-------------------|---------------------------------------------|-----------------------------|-----------------------------|-----------------------------|
|              |                  |                   | Experiment 1                                |                             | Experiment 2                |                             |
|              |                  |                   | Infected                                    | Normal                      | Infected                    | Normal                      |
| Balb/C       | Immune           | Balb/C            | 61.8 $\pm$ 4.2 <sup>c</sup>                 | 27.6 $\pm$ 1.9 <sup>e</sup> | 77.5 $\pm$ 4.2 <sup>d</sup> | 47.0 $\pm$ 3.5 <sup>d</sup> |
|              | Anti- $\theta$ * |                   | ND                                          | ND                          | 40.6 $\pm$ 2.5 <sup>e</sup> | ND                          |
|              | N ascitic*       |                   | ND                                          | ND                          | 90.0 $\pm$ 2.7              | ND                          |
|              | Control          |                   | 42.0 $\pm$ 4.8 <sup>a</sup>                 | 40.5 $\pm$ 5.2 <sup>a</sup> | 49.6 $\pm$ 2.5              | 43.5 $\pm$ 1.6              |
| CBA/H        | Immune           |                   | 42.7 $\pm$ 6.7 <sup>a</sup>                 | 33.7 $\pm$ 5.4 <sup>a</sup> | 32.9 $\pm$ 3.0 <sup>a</sup> | 48.6 $\pm$ 3.9 <sup>a</sup> |
|              | Control          |                   | 28.0 $\pm$ 4.1                              | 40.5 $\pm$ 5.2              | 46.5 $\pm$ 3.7              | 39.7 $\pm$ 4.3              |
| CBA/H        | Immune           | CBA/H             | 69.1 $\pm$ 2.8 <sup>e</sup>                 | 30.9 $\pm$ 3.4 <sup>e</sup> | 72.5 $\pm$ 5.2 <sup>d</sup> | 40.0 $\pm$ 2.9 <sup>d</sup> |
|              | Anti- $\theta$   |                   | ND                                          | ND                          | 44.0 $\pm$ 2.5 <sup>d</sup> | ND                          |
|              | N ascitic        |                   | ND                                          | ND                          | 74.3 $\pm$ 8.4              | ND                          |
|              | Control          |                   | 34.2 $\pm$ 1.1                              | 35.1 $\pm$ 3.7              | 46.5 $\pm$ 3.6              | 44.4 $\pm$ 6.2              |
| Balb/C       | Immune           |                   | 46.2 $\pm$ 3.3 <sup>a</sup>                 | 30.4 $\pm$ 3.8 <sup>b</sup> | 44.0 $\pm$ 2.9 <sup>a</sup> | 41.0 $\pm$ 2.4 <sup>a</sup> |
|              | Control          |                   | 34.9 $\pm$ 5.7                              | 33.7 $\pm$ 5.6              | 40.5 $\pm$ 2.5              | 41.0 $\pm$ 2.4              |

\* Treated with AKR anti- $\theta$  (C3H) ascitic fluid and guinea pig complement, or normal AKR ascitic fluid and guinea pig complement. a, b, c, d, e, Differences by Student's *t* test between values for immune spleen cells treated with anti- $\theta$  ascitic fluid or normal ascitic fluid, immune and control spleen cells overlaid on infected macrophages (infected column), or immune spleen cells overlaid on infected and normal macrophages (normal column). a, *P* > 0.05; b, *P* < 0.05; c, *P* < 0.02; d, *P* < 0.01; e, *P* < 0.001.

ND, Not done.

(Table 3). Comparable levels of specific  $^{51}\text{Cr}$  release from isologous infected macrophages were caused by Balb/C and CBA/H spleen cells (overlaid at 20:1) from mice infected at the same time with the same dose of LCM virus, whereas histoincompatible macrophages were not damaged. Lysis was completely abrogated by treatment with AKR anti- $\theta$  ascitic fluid and guinea pig complement, but not by normal AKR ascitic fluid and complement. Though levels of  $^{51}\text{Cr}$  release from macrophages were more variable than for L cells, probably because of inconsistencies in target cell concentrations and the higher background of non-specific lysis, the effect was both highly significant and repeatable.

The ability of T cells to cause lysis across an allogeneic barrier, when lymphocytes are sensitised to antigens specified by the H-2 gene complex, is well established<sup>12,13</sup>. This also applies to L cells (H-2<sup>k</sup>), which are readily lysed by spleen cells from C57Bl (H-2<sup>b</sup>) mice stimulated in mixed lymphocyte culture by CBA/H (H-2<sup>k</sup>) but not by Balb/C (H-2<sup>d</sup>) lymph node cells. Sufficiently close association for lysis to occur is possible if the T cells is sensitised to alloantigens present on the surface of the target. Interaction between immune lymphocytes and cells expressing antigens expressed by LCM virus is, however, apparently confined to a histocompatible system, perhaps because it is only in this situation that the necessary intimacy of contact is achieved.

This restriction may possibly be overcome by previous treatment of the target population with trypsin. Balb/C (H-2<sup>d</sup>) immune spleen cells will lyse recently trypsinised L cells (H-2<sup>k</sup>) infected with the E-350 strain of LCM virus, the effect being completely abrogated by treatment with a rabbit anti-mouse brain serum cytotoxic for T cells<sup>14</sup>. Our experiments with previously trypsinised WE3-infected L cells have, to date, given equivocal results because of the high background of non-specific  $^{51}\text{Cr}$  release.

An alternative possibility that must be considered in LCM is that the process of virus maturation<sup>5-6</sup> through the cell membrane causes changes in self components, which are recognised only within the syngeneic or semi-allogeneic system. There is ample evidence<sup>15-16</sup>, for instance, that concentrations of H-2 antigens in the cell membrane are decreased in cells productively infected with budding viruses. The cytotoxic T cell may thus be recognising altered self, the implication being that LCM is essentially an autoimmune phenomenon.

These results impose a possible constraint on attempts to demonstrate cytotoxic T cells in infections of man and domestic mammals, where histocompatible cell lines and inbred strains are not available. Perhaps isologous macrophages or lectin-transformed peripheral blood leukocytes may prove

suitable targets in at least some disease states. Restriction of cell-mediated cytotoxicity within a syngeneic or semiallogeneic system may prove a reliable index of T cell involvement in species where the  $\theta$  marker is not available, lysis across this barrier indicating an antibody-associated process<sup>17-19</sup>.

Dr Zinkernagel is supported by the Schweizerische Stiftung Fuer Biologisch-Medizinische Stipendien.

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Received December 10, 1973.

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## Production of colony-stimulating activity by human lymphocytes

THE clonal growth of human bone marrow cells *in vitro* requires the presence of a stimulatory substance(s) designated colony-stimulating activity (CSA)<sup>1,2</sup>. Data available from various clinical and animal experiments suggest that CSA is, or is related to, a physiological controller of granulopoiesis<sup>3,4</sup>. Although the precise biological role of CSA is still uncertain, considerable insights into leukopoietic mechanisms have been gained from the study of interactions of CSA and haemopoietic cells *in vitro*. Two sources of CSA have been identified that stimulate colony formation from human bone marrow: certain foetal tissues and peripheral blood leucocytes<sup>3,4</sup>. The observation that CSA is produced by white cells led naturally to hypotheses concerning the feedback control of stem cells by mature leukocytes<sup>3,5</sup>.

Within the past year, three groups of investigators have fractionated human buffy coat into its component leukocyte subpopulations and identified the monocyte as the principal source of CSA<sup>5-7</sup>. Monocyte-derived macrophages and the human alveolar macrophage also elaborate CSA, whereas neutrophils and unstimulated lymphocytes do not<sup>8</sup>. Recently, however, we examined the effect of mitogens on human lymphocytes and observed that lymphocyte blast transformation was associated with the elaboration of CSA. Lymphocyte-derived CSA stimulated the production of bone marrow colonies containing both granulocytes and mononuclear phagocytes. These observations suggest that granulopoiesis is influenced by humoral substances released from lymphocytes reacting to appropriate stimuli.

Pure populations of neutrophils, monocytes and lymphocytes were separated from peripheral blood as previously described<sup>9</sup>: monocytes and PMN were separated by Ficoll-Hypaque gradients and glass adherence, lymphocytes by removal of other cells adherent to nylon fibre columns. As judged by morphology, peroxidase and alpha-naphthyl butyrase cytochemistry, and lack of phagocytosis, the lymphocyte populations were more than 98.5% pure. Contaminating monocytes were less than 0.1% of the populations—a number insufficient to account for the observed results. Rare nucleated erythrocytes and damaged neutrophils were observed as contaminants. Pure populations of monocytes, neutrophils or lymphocytes were cultured in Leighton tubes at  $1.5 \times 10^6$  ml<sup>-1</sup> in McCoy's medium containing 2 or 15% foetal calf serum and either phytohaemagglutinin-P (PHA, Difco, 35  $\mu$ g ml<sup>-1</sup>), pokeweed mitogen (Grand Island Biological Co., 1:100 dilution), or saline. After 2-6 d the cell-free conditioned medium was collected and tested for CSA after incorporation of 0.05 or 0.1 ml into 0.5% agar underlayers. Normal human bone marrow was tested for its response to this conditioned medium by incorporating  $2 \times 10^5$  cells in 0.3% overlayers<sup>1,5</sup>. After incubation at 37° C for 8-10 d the colonies were counted, smeared and stained for morphological analysis.

As previously described<sup>5,8</sup>, conditioned medium from monocyte preparations had CSA, whereas conditioned medium from unstimulated lymphocytes had no detectable activity. However, incubation of lymphocytes with PHA resulted in detectable stimulatory activity after 3-6 d. The results with pokeweed mitogen were variable until day 6, when low levels of CSA were consistently detected. Table 1 summarizes the results of one experiment which was repeated seven times.

PHA stimulated neither the production or release of CSA by neutrophils nor enhanced monocyte-related CSA. When lymphocytes were incubated in the absence of mitogen and the resultant conditioned medium was later combined with PHA or pokeweed mitogen, no detectable activity resulted. Neither PHA nor pokeweed mitogen—when incorporated directly in agar underlayers—had a detectable direct effect

TABLE 1 CSA activity of conditioned medium from cell cultures.

| Cell type   | Duration of culture (d) | Addition | Colonies per $2 \times 10^5$ marrow cells* |
|-------------|-------------------------|----------|--------------------------------------------|
| Neutrophils | 2-6                     | Saline   | 0-2                                        |
| Neutrophils | 2-6                     | PHA      | 0-2                                        |
| Monocytes   | 2                       | Saline   | $75 \pm 7$                                 |
|             | 5                       | Saline   | $72 \pm 6$                                 |
|             | 5                       | PHA      | $78 \pm 8$                                 |
| Lymphocytes | 2-6                     | Saline   | 0-2                                        |
|             | 3                       | PHA      | $15 \pm 3$                                 |
|             | 4                       | PHA      | $21 \pm 3$                                 |
|             | 6                       | PHA      | $34 \pm 5$                                 |
|             | 3                       | PWM      | $4 \pm 3$                                  |
|             | 6                       | PWM      | $11 \pm 3$                                 |

PWM, pokeweed nitrogen.

\* 0.05 ml of conditioned medium was incorporated in a 0.5 per cent agar underlayer, and  $2 \times 10^5$  normal human bone marrow cells in a 0.3% agar overlayer.

on bone marrow growth. When lymphocytes were incorporated directly in agar with mitogen, many small cell clusters resulted in the underlayer, which obscured evaluation of growth of bone marrow colonies in the upper layer.

The time course of production of lymphocyte CSA in response to PHA is shown in Table 1. Activity was not detectable before day 2, but then increased progressively until day 6. It was detectable when cells were cultivated in 2% foetal calf serum, as well as in the more usual 15%. Both neutrophilic and mononuclear cell colonies developed in bone marrows stimulated by lymphocyte conditioned medium. Occasionally, predominantly eosinophilic colonies were observed (less than 15% of colonies).

Several lines of evidence support the concept that all mammalian haemopoietic cells ultimately arise from a common pluripotent stem cell pool<sup>4,9</sup>. Relatively recent evidence indicates that this is true for lymphoid, as well as non-lymphoid cell lines<sup>10</sup>. The factors controlling differentiation into one or another cell line are poorly understood but almost certainly involve short-range microenvironmental factors as well as systemic hormonal mechanisms<sup>11</sup>. Granulopoiesis and mononuclear phagocyte production *in vivo* are influenced by many stimuli, including bacterial products, antigens and leucocyte removal and administration<sup>3,12-14</sup>.

The data reported here indicate that with mitogen-induced blast transformation, lymphocytes can elaborate a substance(s) able to stimulate the formation of granulocyte and mononuclear cell colonies from human bone marrow *in vitro*. These observations may explain the high levels of CSA observed in mice after antigenic stimulation<sup>14</sup>, and may serve to explain enhanced granulopoietic activity in certain types of humoral and cellular immune reactions. It is not yet known whether lymphocyte CSA is identical to that produced by mononuclear phagocytes. The observation of eosinophilic colonies arising under the stimulus of lymphocyte CSA may provide insights into the intriguing relationship between lymphocytes and the development of an eosinophilic response<sup>15,16</sup>.

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## In vivo localisation of radiolabelled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice

SEVERAL attempts have been made to show the specific localisation *in vivo* of anti-tumour antibodies. Most of these studies, however, either in experimental animals<sup>1,2</sup> or in humans<sup>3</sup> were performed with antibodies obtained by adsorption and elution from poorly characterised crude tumour fractions.

Here we report the specific localisation of <sup>131</sup>I-labelled antibodies against carcinoembryonic antigen (CEA) in heterografts of human colon carcinomas growing in nude mice and show that they can be detected by external scintillation scanning. This model system enables us to test the ability of antibodies directed against a well-characterised human tumour-associated antigen to concentrate in human tumours without performing preliminary experiments in patients.

We have recently shown<sup>4</sup> that CEA as identified by Gold and Freedman<sup>5</sup> was present in heterografts of human colon carcinomas serially transplanted into nude mice (Balb/c

Nu-Nu from G1. Bomholtgard Ltd., Ry, Denmark). CEA was demonstrated on cryostat tumour sections by indirect immunofluorescence using a highly specific rabbit anti-CEA antiserum<sup>6</sup>. The histology of the grafted tumour as well as the localisation of CEA in this tissue was identical to that observed in the primary tumour. Also, CEA extracted from tumour grafts by the perchloric acid method<sup>7</sup> was found by radioimmunoassay<sup>8,9</sup> to be immunologically identical to reference human CEA<sup>6</sup> and, moreover, to be present in similar concentrations in both heterotransplanted and primary tumours. Using a double antibody radioimmunoassay<sup>10,11</sup>, it was possible to detect circulating CEA levels of 20–70 ng ml<sup>-1</sup> in sera of nude mice bearing colon carcinoma tumours larger than 1 g. No CEA was detected in sera of nude mice bearing colon carcinomas smaller than 0.5 g or bearing other human tumours, including breast carcinomas, hypernephromas and melanomas.

Anti-CEA antibodies were isolated by adsorption-elution from a specific immuno-adsorbent prepared in the following way. Eight milligrammes of purified CEA<sup>6</sup> were polymerised with 200 mg of BSA at pH 4.8, using 2 ml of 1% glutaraldehyde solution<sup>12</sup>. Following homogenisation, the polymer was mixed with an equal volume of Sephadex G-25 and packed in a 1 cm diameter column. One ml of hyperimmune goat anti-CEA antiserum which exhibited little reactivity with the non-specific cross-reacting antigen (NCA)<sup>13–15</sup>, was run through the column at a flow rate of 3 ml h<sup>-1</sup>. The adsorbed antibodies were eluted with 3 M KSCN<sup>16</sup>, dialysed against 0.01 M Tris-HCl buffer, pH 7.5, and concentrated to 1 ml. This antibody fraction contained 2 mg of protein and was able to bind 50% of 1 ng of CEA at a dilution of 1:70,000 when titrated in the radioimmunoassay.

A hundred microgrammes of the antibody fraction were labelled with 2 mCi of <sup>131</sup>I using the lactoperoxidase method<sup>17</sup>, mixed with 1 ml of normal goat serum and filtered on a Sephadex G-200 column. The 7S antibody fraction was collected and used immediately for the localisation experiments. About 60–70% of the radioactive material in the 7S fraction was specifically bound to the CEA immuno-adsorbent. For control experiments, normal goat 7S globulins, treated with 3 M KSCN, were labelled with <sup>125</sup>I and further purified on Sephadex G-200.

Volumes of 0.2 ml of the 7S fraction containing about 2 µg of radioactive antibody (specific activity: 8 µCi µg<sup>-1</sup>) and

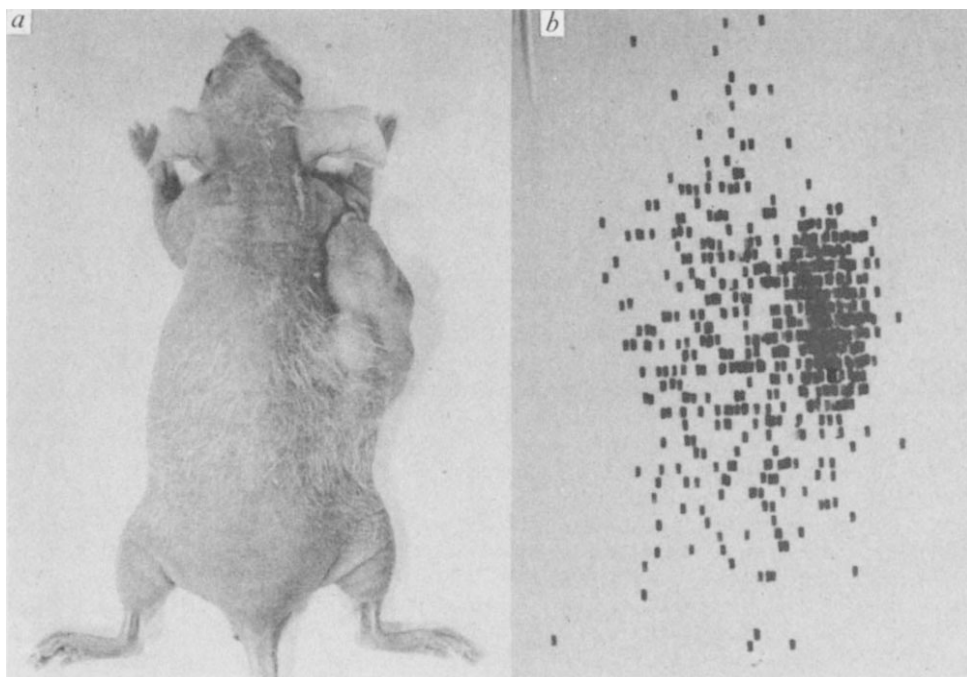


Fig. 1 *a*, Nude mouse bearing a heterograft of human colon carcinoma is shown in the scanning position. *b*, The total-body scan from the same mouse obtained 3 d after injection of 2 µg <sup>131</sup>I labelled anti-carcinoembryonic antibodies (dose of radioactivity injected = 16 µCi).



TABLE 1 Comparison of antibody and normal 7S concentration in tumour liver and muscles

| Type and weight of tumours | Protein Injected | Tumour % per g* | Liver % per g* | Muscle % per g* | Tumour: liver ratio† | Specificity index† | Tumour: muscle ratio† | Specificity index† |
|----------------------------|------------------|-----------------|----------------|-----------------|----------------------|--------------------|-----------------------|--------------------|
| Exp. I                     | Antibody         | 17.3            | 4.9            | 2.0             | 3.5}                 | 4.1                | 8.7}                  | 4.5                |
| Co 115 1.620 g             | N 7 S            | 4.6             | 5.3            | 2.4             | 0.9}                 |                    | 1.9}                  |                    |
| Exp. II                    | Antibody         | 24.5            | 4.4            | 1.2             | 5.6}                 | 2.7                | 20.4}                 | 2.4                |
| Co 115 1.960 g             | N 7 S            | 13.4            | 6.4            | 1.6             | 2.1}                 |                    | 8.4}                  |                    |
| Exp. III                   | Antibody         | 23.4            | 2.6            | 2.8             | 9.0}                 | 3.9                | 8.4}                  | 4.1                |
| Co 115 2.200 g             | N 7 S            | 13.2            | 5.7            | 6.5             | 2.3}                 |                    | 2.0}                  |                    |
| Exp. IV                    | Antibody         | 24.3            | 6.9            | 7.2             | 3.5}                 | 3.5                | 3.4}                  | 3.5                |
| Co 115 0.575 g             | N 7 S            | 12.4            | 12.4           | 12.8            | 1.0}                 |                    | 1.0}                  |                    |
| Exp. V                     | Antibody         | 15.0            | 10.0           | 1.8             | 1.5}                 | 1.9                | 8.3}                  | 1.8                |
| Co 111 0.495 g             | N 7 S            | 7.6             | 9.4            | 1.6             | 0.8}                 |                    | 4.8}                  |                    |
| Exp. VI                    | Antibody         | 12.9            | 6.3            | 1.7             | 2.0}                 | 2.3                | 7.6}                  | 2.1                |
| Co 112 0.405 g             | N 7 S            | 8.5             | 9.9            | 2.3             | 0.9}                 |                    | 3.7}                  |                    |
| Exp. VII                   | Antibody         | 7.4             | 3.9            | 1.8             | 1.9}                 | 1.2                | 4.1}                  | 1.0                |
| El 4 0.510 g               | N 7 S            | 8.9             | 5.5            | 2.1             | 1.6}                 |                    | 4.2}                  |                    |

\* Concentration of either antibody or normal 7S globulin (N 7S) expressed as % of the total specific radioactivity per g of tissue.

† Tumour: liver or tumour: muscle ratio are obtained by dividing the tumour concentration of either antibody or normal 7S globulin by the organ concentration of each of these proteins.

‡ Specificity indices are obtained by dividing the tumour: normal organ concentration ratio of antibody by that of normal 7S globulin.

200  $\mu$ g of normal goat 7S globulins were injected intravenously into nude mice bearing subcutaneous grafts of human colon carcinomas. At different time intervals ranging from 2 h up to 3 d after injection, mice were scanned with a 3 inch crystal photoscanner (Picker Magnascanner 500). During the scanning procedure, the mice, anaesthetised by intraperitoneal injection of phenobarbital, were immobilised in the prone position as shown in Fig. 1a. After 2–6 h, the radioactivity was homogeneously distributed throughout the mice with no apparent localisation in the tumours. No difference could be detected between tumour-bearing mice and normal controls. After 1 d, however, the radioactivity began to localise in the tumour area and gave an optimal detectable contrast by day 3 (Fig. 1b). At that time, the mice were killed and scanned again after separating the tumour from the body. This last scanning confirmed that the major radioactive site was related to the tumour and not to the adjacent liver.

After complete dissection of the animal, each organ and pieces of the body were weighed and placed in well-type scintillation counter. For the 24 g mouse seen in Fig. 1, it was found that 40% of the total radioactivity recovered was concentrated within the 1.8 g tumour. Several other scanning pictures showing clear tumour localisation were obtained with different mice bearing variously sized heterografts, all derived from the same colon carcinoma (Co 115). The smallest tumour nodule detectable by scanning weighed 200 mg. Grafts of two other human colon carcinomas (Co 111 and Co 112), with more differentiated histology and less abundant stroma and vascularisation<sup>4</sup> than Co 115, showed a lower specific concentration of antibodies (see Table 1) which were not detectable by scanning.

To take into account the possible non-specific accumulation of proteins in the extravascular space and necrotic regions of the tumour<sup>18</sup>, the antibody localisation was studied by simultaneous injection of <sup>131</sup>I-labelled antibodies and <sup>125</sup>I-labelled normal goat 7S globulins (N 7S). In two experiments, the labels were reversed. The average values of four representative experiments performed in mice bearing Co 115 tumour are presented in Fig. 2. The results are expressed in percentage of the total radioactivity recovered per g of each tissue studied. The concentration of antibodies in the tumour was more than two-fold greater than N 7S whereas in normal tissues the antibodies had lower values than the control protein.

In Table 1 the individual results from seven different experiments are summarised. Concentration values of labelled antibodies and N 7S observed in tumour, liver and muscle

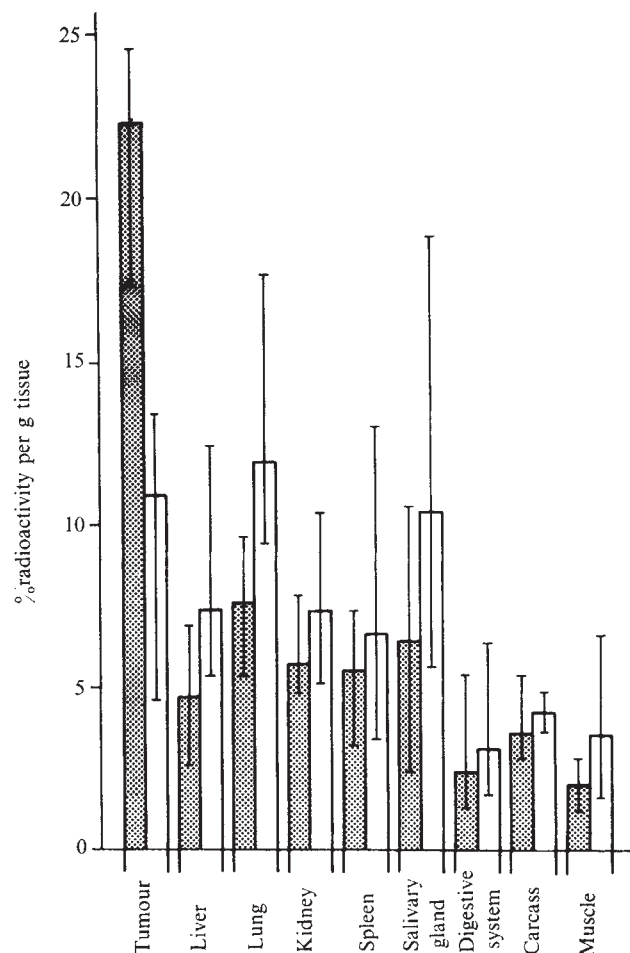


Fig. 2 Distribution of goat anti-CEA antibodies and normal goat 7S globulins in nude mice bearing heterografts of human colon carcinoma (Co 115). Three days after simultaneous injection of both proteins each labelled with a different iodine radioisotope (as described in the text), the tumour and different tissues were weighed and then measured in a dual channel well-type scintillation counter. Bar graphs represent the average value of four different experiments (% of total specific radioactivity recovered per g of tissue) for antibodies (shaded area) and for normal goat 7S globulin (open area). The solid lines represent the range of values.

can be compared. For both radioactive preparations, the ratio of tumour:normal organ concentration was calculated. A specificity index was obtained by dividing the concentration ratio of antibodies by that of N 7S. This index was significantly positive in all colon carcinomas tested, including Co 111 and Co 112 (experiments V and VI), which were not detectable by scanning. In contrast, when the same experiments was performed in C57Bl/6 mice bearing a transplanted EL4 solid lymphoma (experiment VII), the specificity indices were not significant.

Evidence was obtained that the specificity of antibody localisation was decreased with the presence of necrotic tissue within the tumour. In experiment II, the tumour exhibited a significant degree of central necrosis. Despite a high absolute concentration of antibodies in this tumour, relatively low specificity indices were observed compared with those obtained for less necrotic tumours (experiments I, III and IV). Furthermore, when the radioactivity of a small non-necrotic fragment selected from the tumour of experiment II was measured, the tumour:liver ratio of antibody was 14.0 and the specificity index reached 8.6 (the highest value yet observed). In three other experiments, cells from disrupted tumour fragments were counted after several washes with buffered saline. It was found that about 55% of the antibody radioactivity remained bound to the cells compared with 15% of the N 7S radioactivity.

It should not be concluded from these experimental studies that the injection of patients with labelled heteroanti-CEA antibodies will, in any case, enable the scanning detection of CEA-containing cancers. The major difficulties in humans would be the relatively large amounts of circulating CEA<sup>8,9,11</sup>, as well as the presence of small quantities of CEA in non-malignant tissues<sup>6,19-21</sup>. Further work is needed to assess whether the use of highly specific antibodies directed against CEA may open a new dimension in the field of tumour localisation by radioactive tracers.

We thank Professor H. Isliker for advice and suggestions, Dr B. Scazziga, Miss L. Cuendet and Miss R. Kollman, Thyroid Function Unit, Department of Medicine, University, of Lausanne, for assistance in radioactive scanning. This work was supported by grants from the Ludwig Institute for Cancer Research and the Swiss National Foundation for Scientific Research.

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Received November 8, 1973.

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## Immunological detection of antigen(s) associated with rat colon carcinoma

CANCER of the colon and rectum is exceeded only by cancer of the lung as a cause of cancer death in the United States. Of the approximately 350,000 deaths from cancer in 1973, 47,400 were from cancer of the colon and rectum; of the approximately 665,000 cancers diagnosed in 1973, 79,000 were in the colon and rectum. Denmark, Great Britain, Ireland, Canada, New Zealand, Australia, Belgium and Austria have equally high or even slightly higher death rates from this disease and it is a significant cause of morbidity and mortality in most western countries. Improved surgical techniques and the development of new diagnostic methods have not significantly affected these mortality statistics<sup>1</sup>.

A useful animal model for the study of human colon carcinoma is the rat bearing adenocarcinomas of the colon induced by 1,2-dimethylhydrazine (DMH)<sup>2,3</sup>. Six to 8 months after initiation of treatment, 60 to 100 per cent of rats have one or more colon tumours which cause bleeding, intussusception or obstruction. The tumours are visible through the thin wall of the colon and are readily accessible for surgical or other treatment. Methods for early detection of tumours are needed for (a) development and evaluation of preventive or therapeutic measures and (b) studies of tumour development under dietary or other regimens designed to retard or enhance colon carcinogenesis<sup>3-5</sup>.

Human colon carcinomas produce a glycoprotein, carcinoembryonic antigen (CEA)<sup>6</sup>, which is detectable in serum<sup>7</sup> or plasma<sup>8</sup>. Although CEA is not specific for colon tumours, detection and quantitation of circulating quantities may be useful in the diagnosis of colon and other cancers, for prognosis after surgical resection and for monitoring chemotherapy<sup>9</sup>. We have detected antigen(s) in DMH-induced rat colon carcinomas using antisera produced in rabbits (see below). Since the antigen(s) was found in the rat tumours but not in normal rat colon it may provide a rat model for CEA in human colon carcinoma.

Identification of the tumour-associated antigen analogous to human CEA, in the rat model described above would facilitate study of (1) the relationship between the blood level of antigen and size or other anatomic characteristics of colon tumours, (2) response of tumours to treatment, and (3) experimental induction and development of tumours.

Twenty-eight male, 4-week-old Sprague-Dawley rats (Charles River Laboratories) were fed a semisynthetic diet for 3 months, given ten weekly intragastric doses of DMH (30 mg kg<sup>-1</sup> in 0.9% NaCl) and killed when they developed signs of tumour in the intestine or ear duct. Then control rats were given 0.9% NaCl. At autopsy, colon tumours 3



mm or greater in diameter and sections of normal colon (starting 1 cm away from the tumour) were frozen in liquid nitrogen. A small section of tumour was fixed in 10% neutral buffered formalin and processed for histological examination.

Pooled tumours (4.2 g) were minced and homogenized for 1 h at 0° C in three volumes of 0.9% NaCl using a motor-driven Teflon pestle. The homogenate was centrifuged at 500 g and 4° C for 30 min; the supernatant was dialysed against distilled water for 60 h with fifteen 4-h changes. The non-dialysable portion was flash frozen in methanol-dry ice and lyophilized to dryness to yield 186 mg of product. Extracts were prepared, by the same procedure, from 4.9 g of normal colon from ten control rats and 5.3 g of normal colon from tumour-bearing rats. Portions of the three extracts were dissolved separately in 0.9% NaCl at a concentration of 4 mg ml<sup>-1</sup>.

Antibodies to the tumour macromolecules were produced by giving New Zealand adult rabbits three weekly intramuscular injections of a mixture of 0.25 ml of tumour extract and 0.75 ml of aqueous aluminum hydroxide gel as an adjuvant<sup>10</sup>. The rabbits were bled 7 d after the last injection, injected with the tumour extract-aluminum hydroxide gel mixture and bled repeatedly for several weeks.

The blood was allowed to clot at room temperature for 1 h, held at 4° C overnight and centrifuged at 280 g and 4° C. Sera were decanted and at 56° C for 30 min. Antibodies to tumour-associated antigens and normal rat tissue components were found in the antisera by double immunodiffusion (Ouchterlony) and counterimmunoelectrophoresis techniques on rehydratable Agarose media. Heterologous antibodies were removed from the antisera by absorbing with (a) extracts of normal rat colon (1 part to 100 parts of the antisera) and (b) affinity chromatography beads (CNBR-Sepharose, Pharmacia) to which normal rat colon macromolecules were coupled. Absorption was continued until the antisera did not react with normal colon components when assayed by the Ouchterlony technique. Two-fold serial dilutions of normal colon extracts were tested with the absorbed antisera to confirm that negative reactions were not due to formation of soluble immunocomplexes in the prozones of the antisera.

Absorbed antisera to rat colon tumours (10 µl) were reacted by the Ouchterlony technique with 10 µl of extracts of tumour, normal rat colon and normal colon from tumour-bearing rats for 48 h in a humid chamber at room temperature. Reaction was obtained only with extracts of tumour. Counterimmunoelectrophoresis was performed on prototype 35 mm Agarose strips (45 mA, 30 V, 60 min) in which macromolecules from the colon tumours and normal colon were electrophoretically transported to the absorbed tumour antibodies<sup>10</sup>. The same concentrations were used as in the Ouchterlony technique. This more sensitive technique supported the Ouchterlony results. When seven two-fold serial dilutions of colon tumour extract and of normal colon extract were reacted with a constant concentration of antibody to rat colon tumours, reactions were obtained with the tumour extract at all dilutions, but no reactions were obtained with dilutions of the normal colon extracts.

Preliminary work indicated that the absorbed antisera reacted with rat foetal extracts. Forty fetuses taken from four Sprague-Dawley rats on the fourteenth day of gestation were dissected free of maternal tissues, decapitated and their tails docked. Pooled foetuses gave 2.8 g of tissue from which a crude saline extract was prepared by homogenization and centrifugation. Counterimmunoelectrophoresis performed as described above indicated a strong reaction between foetal extract components and the tumour antibodies. Preliminary results indicate that the tumour antibodies do not cross-react with human CEA and that rat tumour antigen does not react with anti-CEA antisera. The difference may be one of species specificity as was found with  $\alpha$ -foetoprotein produced by human or rat hepatocarcinomas<sup>11</sup>.

A. B.-K. G. carried out this work in partial requirement for the MS degree.

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## Involvement of amino and sulphydryl groups in olfactory transduction in silk moths

ROZENTAL and Norris<sup>1</sup> found evidence of a chemosensory mechanism of olfaction and gustation in the cockroach *Periplaneta americana* that involves the formation of disulphide bonds. I wish to report that olfactory transduction in silk moths does not seem to involve disulphide links, although sulphydryl and amino groups are important.

Experimental evidence on mechanisms of molecular interaction with chemoreceptors might aid the choice among different theories of taste and olfaction<sup>2-8</sup>, and the silk moths are useful models for such work because the antennae of the males contain cells specialised to capture chemical information from a particular molecular species<sup>9-15</sup>. I have perfused amputated male antennae of the silk moths *Antheraea pernyi* and *Bombyx mori* with solutions of chemical reagents selective for protein side chains. (Previous control perfusions were performed with the specific solvents for the reagents.) Olfactory responses were measured by the electroantennogram method<sup>9,14</sup>. Unlike that of *B. mori*, the sex pheromone of *A. pernyi* is not yet available, and so I prepared an extract from the female glands<sup>12</sup>.

2,3-Dimethylmaleic anhydride (DMA) and 2-methylmaleic anhydride (citraconic anhydride, CA) are specific blocking agents for amino groups of proteins<sup>16</sup>, but their action is reversible by decreased pH. Olfactory responses were inhibited with DMA, pH 7.5, at a threshold concentration of 2 mM. On re-perfusion with control Ringer solution, pH 4.8, there was substantial reversal of inhibition. Figure 1a shows the electroantennogram responses before, during and after the reversal of inhibition by 4.7 mM DMA. Similar reversible inhibition was obtained with CA. There was also inhibition with  $\geq 0.9$  mM 2,4,6-trinitrobenzenesulphonic acid<sup>17</sup>.

Behavioural and other studies have suggested that sulphhydryl groups are involved in taste and olfaction in various insects<sup>18-20</sup> and man<sup>21</sup>. To test for such involvement in *A. pernyi*, I perfused antennae with N-ethylmaleimide (NEM) at pH 7.5. Inhibitions graded according to concentration were found (Fig. 1b). *p*-Chloromercuribenzoate was inhibitory at a threshold of 0.2 mM; appreciable reversal was achieved by re-perfusion with 1.0 mM reduced glutathione. Mersalyl (salyrganic acid) and iodoacetic acid inhibited, with thresholds of approximately 1 mM and 0.1 mM respectively. I found similar, though less strong indications that amino and sulphhydryl groups are involved in the olfactory process of *B. mori*; the antennae are less robust in successive perfusions than the larger antennae of *A. pernyi*.

The exact site of action of an inhibitor on a whole antenna is unknown, and rather than the receptor macromolecule, ion-channels or an ion-pump mechanism, for example, might be

impaired. The latter is less likely, for standing potentials<sup>22</sup> that I measured tended to be maintained after perfusion with inhibitors. The unambiguous identification of both the inhibitor target site and the modified groups, however, requires isolation of the receptor and the inhibition products.

The relatively high concentration needed for inhibition could indicate non-selectivity of action for the amino and sulphhydryl group reagents. Since diminished olfactory responses in *A. pernyi* did not change appreciably for almost 1 h after perfusion with the inhibitors, equilibrium may have been reached. But if the resistance to diffusive flux were high, because of a hidden site of action for example, the variation of concentration gradient with time would not be appreciable, thus giving rise to quasi-stationary diffusion<sup>23</sup>. If so, the concentration of inhibitor at the binding-sites would be somewhat lower than in the perfusing system.

The next question I considered was whether disulphide groups might be involved in silk moth chemosensory transduction as they seem to be in the function of vertebrate acetylcholine receptors<sup>24-27</sup> and taste and olfaction in *P. americana*<sup>1</sup>. Antennae of *A. pernyi* were perfused with reduced glutathione, L-cysteine, dithioerythritol (DTE)<sup>28</sup> and  $\beta$ -mercaptoethanol. Inhibition was found only at concentrations greater than approximately 10 mM, and it was not reversible, suggesting non-specificity of action. Below 1 mM there was no detectable effect; from 1 mM to 10 mM responses were slightly activated; with DTE there was some activation even at 30 mM. A possible explanation for this activation is that receptor sulphhydryl groups were protected by the reducing agents. Similar results were obtained with *B. mori*.

A tentative conclusion is that, in contrast to findings with *P. americana*, interconversion between -SH and S-S<sup>1,29</sup> is not characteristic of olfactory transduction in *A. pernyi* and *B. mori*.

I thank the Deutsche Forschungsgemeinschaft for financial support, Miss Carola Hahn for technical help and Drs D. Schneider and H. Dannenberg for facilities.

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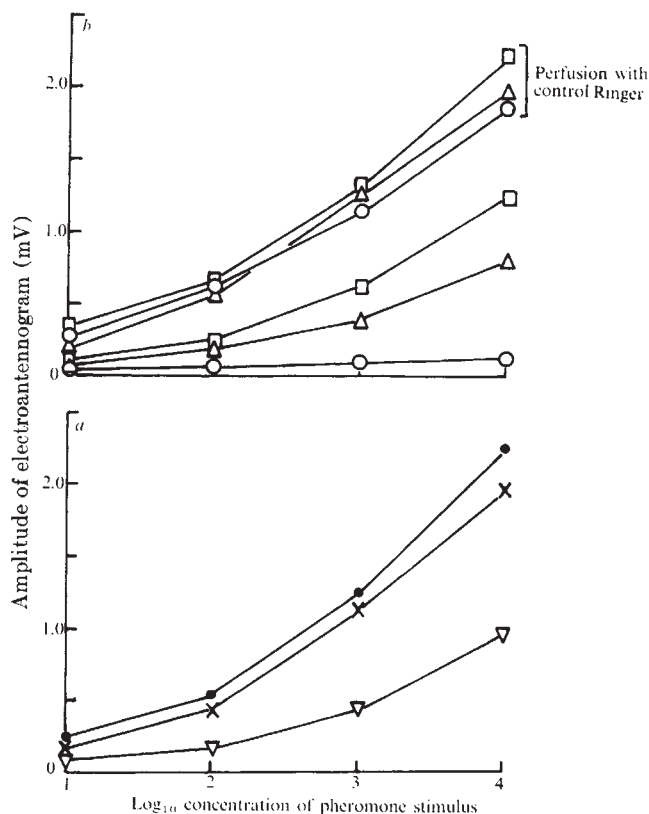


FIG. 1 Olfactory responses (amplitude of electroantennogram) of *A. pernyi* male antennae after perfusion with reagents for (a) amino and (b) sulphhydryl groups of proteins. Since the concentration of pheromone in the extract of pheromone gland (see text) is unknown, the concentration of the stimulus is expressed in arbitrary units. a: ●, Control; ▽, 4.7 mM DMA at pH 7.5; ×, reversal of inhibition by re-perfusion with control Ringer adjusted to pH 4.8. b, Perfusions with various concentrations of NEM: □, control, followed by 0.1 mM NEM; △, control, then 1.0 mM NEM; ○, control, then 10.0 mM NEM. Data represent mean values for three antennae; the experimental variability was approximately  $\pm 0.1$  mV.



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## The $\alpha$ 1-acute phase protein response in rats as a possible indicator of the relative smoking risks of different cigarettes

AN increase in the serum levels of the  $\alpha$ 1-acute phase protein, indicative of local tissue damage<sup>1</sup> has been shown in rats exposed to cigarette smoke for periods of up to 30 d. The increases correlated well with the degree of damage to the respiratory system as evidenced by the pathological changes observed *post mortem* in the lungs (L.A.E., T.E.B., D. A. Darcy and J. P. O'Sullivan, unpublished).

The greatest increases were in the rats exposed to the smoke of cigarettes made of flue-cured tobacco, which are by far the most popular type of cigarettes smoked in Britain. Considerable increases in the  $\alpha$ 1-protein were also seen in rats exposed to the smoke of cigarettes made mainly from air-cured, unfermented tobacco, a type popular in France, Spain and other European countries. Rats exposed to the smoke of cigarettes made of air-cured fermented cigar tobacco, however, showed only a slight increase in their serum  $\alpha$ 1-acute

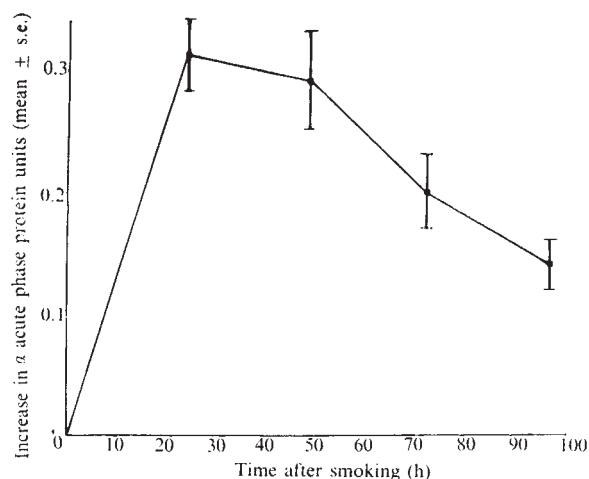


FIG. 1 The effect of smoking 35 cigarettes on rat serum  $\alpha$ 1-protein.

$\alpha$ 1-Acute phase protein determinations were carried out by the method of Darcy<sup>2,3</sup> on 0.2 ml blood taken from a tail vein. Groups of five rats were then exposed to the smoke from 30 to 40 cigarettes in a Martin Wright smoking machine<sup>4</sup>. The machine was set to give a 35 ml puff of 2 s duration and the smoking period lasted 3 to 4 h. The animals were again bled for  $\alpha$ 1-acute phase determination at a fixed time, usually 24 h after the end of their smoking period. Male rats, of the Wilkie hooded strain, aged between 10 and 20 weeks bred under minimal disease conditions were used. Control animals were sham smoked.

Figure 1 shows the time response curve to the smoke of 35 commercial English cigarettes (made of flue-cured Virginia tobacco). The optimum response appears to be

TABLE 1 Effect of different tobacco smokes on serum  $\alpha$ 1-acute phase protein in rats.

| Tobacco type                              | Country     | Sugar content (%) | pH of smoke | Tar T.P.M. (mg) | Nicotine (mg) | No. of cigarettes smoked | Increase in $\alpha$ 1-acute phase protein units ( $\pm$ s.e.)* |
|-------------------------------------------|-------------|-------------------|-------------|-----------------|---------------|--------------------------|-----------------------------------------------------------------|
| Flue-cured cigarette                      | England     | 17.8              | 4.0         | 21.8            | 2.2           | 40                       | 1.10 $\pm$ 0.06†                                                |
|                                           | England     | 17.8              | 4.0         | 21.8            | 2.2           | 35                       | 0.97 $\pm$ 0.09                                                 |
|                                           | England     | 18.0              | 4.15        | 21.1            | 2.3           | 40                       | 0.78 $\pm$ 0.05                                                 |
| Air-cured cigarette                       | France      | 2.0               | 8.2         | 18.6            | 2.5           | 30                       | 0.44 $\pm$ 0.08                                                 |
|                                           | France      | 2.0               | 8.2         | 18.6            | 2.5           | 30                       | 0.29 $\pm$ 0.01                                                 |
|                                           | Switzerland | 1.6               | 6.0         | 16.3            | 2.1           | 35                       | 0.44 $\pm$ 0.04                                                 |
|                                           | Spain       | 0.5               | 8.6         | 17.5            | 2.6           | 40                       | 0.27 $\pm$ 0.05                                                 |
| Air-cured cigar                           | U. States   | 0.4               | 8.7         | 27.5            | 5.8           | 30                       | 0.54 $\pm$ 0.01                                                 |
|                                           | U. States   | 0.4               | 8.7         | 27.5            | 5.8           | 30                       | 0.24 $\pm$ 0.02                                                 |
| Air-cured, fermented cigar                | U. States   | 0.03              | 9.0         | 23.5            | 3.5           | 30                       | 0.19 $\pm$ 0.02                                                 |
|                                           | U. States   | 0.03              | 9.0         | 23.5            | 3.5           | 30                       | 0.05 $\pm$ 0.01                                                 |
| Control rats not exposed to tobacco smoke |             |                   |             |                 |               |                          | 0.03 $\pm$ 0.02                                                 |
|                                           |             |                   |             |                 |               |                          | 0.02 $\pm$ 0.01                                                 |

\* Mean baseline for all rats 0.19  $\pm$  0.01.

† Mean of ten rats.

phase protein. This could be of significance in relation to the relatively low lung cancer mortality of cigar smokers compared to cigarette smokers. As the acute phase proteins usually appear in increased concentration in the blood within a few hours of inflammation or tissue injury, it seemed possible that the  $\alpha$ 1-acute phase protein response in the rat could offer a convenient rapid method of comparing deleterious effects and possible smoking risks of different cigarettes. We report here preliminary observations.

between 24 and 48 h, and a 24-h interval between the end of the smoking period and determination of the  $\alpha$ 1-acute phase protein response was selected for comparison of the effect of the smokers of different tobaccos shown in the table.

Although only limited inferences should be drawn from these preliminary results, the smoke of cigarettes made from flue-cured, high sugar content tobacco seems to produce the greatest increases in  $\alpha$ 1-acute phase protein. Increases to between five and seven times the control (presmoking) levels

were observed. The smoke of air-cured tobacco cigarettes and of cigarettes made from unfermented cigar tobacco produced increases of up to 3.5 times the control value. The lowest increases, to less than twice the control values were found in animals exposed to the smoke of cigarettes made from air-cured fermented cigar tobacco. This agrees with the findings of our longer-term experiments, and is consistent with the much lower risk of lung cancer in cigar smokers compared with cigarette smokers.

The effect on the serum  $\alpha$ 1-acute phase protein does not seem to bear any direct relation to the tar and nicotine content of the smoke. There may be a possibility that the acid nature (pH 4) of the smoke of the high sugar content, flue-cured tobacco<sup>5</sup> makes some contribution to the intensity of the  $\alpha$ 1-protein response.

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## Synthetic insecticide with a new order of activity

WE describe a new insecticide, related to the pyrethrins, and considerably more active than any other available compound, of whatever class ( $LD_{50}$  about 0.0003  $\mu$ g per insect, equivalent to about 0.03 mg  $kg^{-1}$ ).

Recently, we synthesised insecticidal esters of 5-benzyl-3-furylmethyl alcohol<sup>1</sup> and 3-phenoxybenzyl alcohol<sup>2,3</sup> with (1R, *trans*) 2,2-dimethyl-3-(2,2-dihalovinyl)cyclopropane carboxylic acids. (The nomenclature adopted here is a convenient alternative to that based on the sequence rule<sup>4</sup> where, for example, the dihalovinyl acids formally derived from (+)-*trans* (1R, 3R) chrysanthemic acid by replacement of the vinyl methyl groups would be (1R, 3S)). The ( $\pm$ )-*trans*-dibromovinyl ester of 5-benzyl-3-furylmethyl alcohol has also been reported<sup>5</sup>. We have now prepared the corresponding (1R, *cis*) acids and esterified them with appropriate alcohols, including those of the  $\alpha$ -ethynyl- (Belgian Patent 738112 to Badische Anilin-Soda-Fabrik Aktiengesellschaft and Ger. offen. 2,230,862 to Sumitomo Chemical Company Limited) and  $\alpha$ -cyanobenzyl (Ger. offen. 2,231,312 to Sumitomo Chemical Company Limited) series. (1R, *cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylic acid (Fig. 1b) was synthesised as shown *via* the aldehyde (Fig. 1a) derived from methyl (+)-*cis* (1R, 3S)-chrysanthemate by an established method of ozonolysis (French Patent 1,580,474 to Roussel-Uclaf, S.A.). The ester (NRDC 156) with 3-phenoxybenzaldehyde<sup>6</sup> cyanhydrin (( $\pm$ )- $\alpha$ -cyano-3-phenoxybenzyl alcohol) (Fig. 1d) was very active as an insecticide. A solution of it in hexane deposited crystals after cooling for 2 months. Recrystallisation gave a single pure isomer (melting point 100°C, sharp; mass spectrometric molecular weight, 503; Br = 79) with an NMR spectrum showing only one signal for the CH(CN) proton of  $\tau$ 3.64, whereas the parent mixture of isomers showed two signals of equal intensity at  $\tau$ 3.64 and  $\tau$ 3.72. The mother liquors contained material giving 2 signals (relative areas 1:4) also at  $\tau$ 3.64 and  $\tau$ 3.72.

TABLE 1 Toxicity of various insecticides to insects

| Compound                                                    | Relative toxicity* to                      |                                                       |
|-------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------|
|                                                             | Houseflies<br>( <i>Musca domestica</i> L.) | Mustard beetles<br>( <i>Phaedon cochleariae</i> Fab.) |
| Pyrethrin I                                                 | 2.0                                        | 160                                                   |
| Bioresmethrin (NRDC 107)                                    | 100†                                       | 100‡                                                  |
| Cismethrin (NRDC 119)                                       | 41                                         | 52                                                    |
| K-o-thrin (RU 11,679)                                       | 130                                        | 170                                                   |
| Parathion                                                   | 37                                         | 7                                                     |
| DDT                                                         | 4-15                                       | 1.1                                                   |
| Dieldrin                                                    | 35                                         | 4-10                                                  |
| ( $\pm$ )- $\alpha$ -cyano-3-phenoxybenzyl ester (NRDC 156) | 1000                                       | 1000                                                  |
| NRDC 161 (crystalline component of above; see Fig. 1e)      | 2300                                       | 1600                                                  |
| Material in mother liquors from above crystallisation       | 350                                        | 380                                                   |

\* By topical application of acetone solutions as described previously<sup>1</sup>, w/w basis.

†  $LD_{50} \sim 0.006 \mu$ g per insect

‡  $LD_{50} \sim 0.005 \mu$ g per insect

Table 1 shows that the crystalline isomer (NRDC 161) was outstandingly active against insects compared with the non-crystalline form and with other insecticides. To mustard beetles, on a molar basis, NRDC 161 is twenty-four times as active as bioresmethrin<sup>7,8</sup> (5-benzyl-3-furylmethyl (+)-*trans*-chrysanthemate, molecular weight 338) and fifteen times as active as pyrethrin I, the principal constituent of pyrethrum extract<sup>8</sup>. To a normal strain of houseflies NRDC 161 is thirty-four times as active per mol as bioresmethrin (itself more potent than most insecticides) and 1,700 times as active as pyrethrin I. The activity against houseflies was further enhanced by pretreatment (2  $\mu$ g per insect) with the synergist sesamex, which decreased the  $LD_{50}$  value from 0.00034 to 0.00018  $\mu$ g per insect ( $\sim 0.002$  mg  $kg^{-1}$ ) (synergistic factor = 18, close to the value for less active synthetic pyrethroids<sup>9</sup>). The  $LD_{50}$  values for *A. stephensi* (ng per female) and *G. austeni* (ng per mixed sex) are respectively 0.036 and 0.08, giving an activity about forty-five times per mole that of bioresmethrin (F. Barlow, personal communication). The insecticidal activity of NRDC 161 and the effects of synergists are being studied further.

To obtain information about the absolute configuration of NRDC 161, (+)- $\alpha$ -cyano-3-phenoxybenzyl alcohol (optical purity, 95% from NMR spectrum of ester below) was prepared from 3-phenoxybenzaldehyde (Fig. 1c) and hydrogen cyanide in the presence of D-oxynitrilase<sup>10</sup> (conveniently available in emulsin preparations, such as  $\beta$ -glucosidase, from Koch-Light Laboratories Ltd) and esterified with (1R, *cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylic acid as in Fig. 1. The ester was not crystalline and resembled, in properties and insecticidal activity, material from the mother liquor after separating the crystalline isomer. If the (+)-cyanohydrin thus obtained from 3-phenoxybenzaldehyde has the same configuration as the D-(+)-mandelonitrile derived from benzaldehyde via the oxynitrilase<sup>10</sup>, the absolute configuration of the  $\alpha$ -cyano-3-phenoxybenzyl alcohol in the potent crystalline isomer is S, for (+)-mandelonitrile is known to give (–)-mandelic acid, related to D-(+)-R-glyceraldehyde<sup>11</sup>. The potent crystalline isomer,  $[\alpha]_D + 15.6^\circ$ , is therefore tentatively assigned the structure [S]- $\alpha$ -cyano-3-phenoxybenzyl *cis*-(1R,3R) 2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate (Fig. 1e) pending determination of the absolute configuration by the X-ray examination (now in progress by Dr J. D. Owen and Dr M. R. Truter).

The discovery of a pyrethroid with such great toxicity to insects and atypical activity in mammals (see accompanying



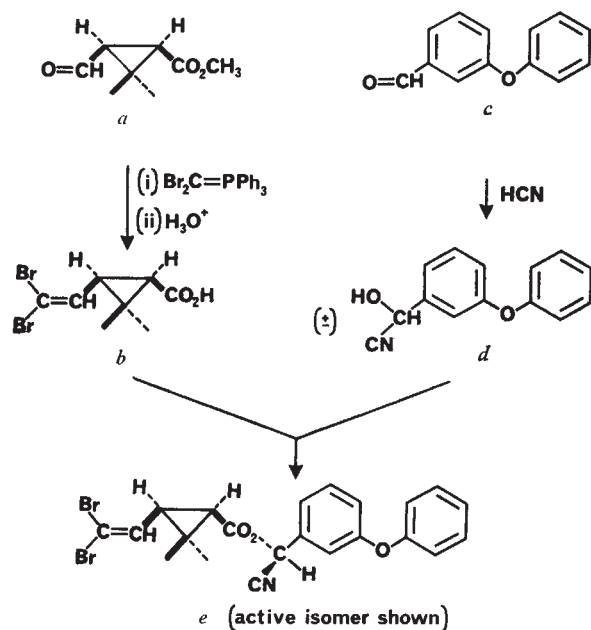


FIG. 1 Synthesis of the active compound.

paper<sup>12</sup>) has important theoretical and practical implications, now being examined.

The new compounds are protected by UK Patent Applications Nos 20539/73, 39539/73 and 49098/73 and corresponding foreign applications.

We thank Dr J. Martel (Roussel Uclaf SA) for a generous gift of (+)-*cis*-chrysanthemic acid, Dr J. M. Barnes, Mr R. D. Verschoyle and Mr F. Barlow for results and discussions, and the National Research Development Corporation for financial support.

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Received December 28, 1973.

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Given as solutions in glycerol formal by the intravenous route, the lethal dose of NRDC 156 was 6 to 8 mg kg<sup>-1</sup> and of NRDC 161, 2 to 2.5 mg kg<sup>-1</sup>. Orally, as solutions in arachis oil, an LD<sub>50</sub> could not be computed, but the rats showed severe toxic effects, with some deaths in the dose range 80 to 160 mg kg<sup>-1</sup> for 156 and 25 to 63 mg kg<sup>-1</sup> for 161.

The signs of poisoning in these rats were not the same as those described for other pyrethroids<sup>2</sup>. The first sign was excessive salivation without lachrymation, rapidly followed by continuous irregular jerking movements of the limbs, progressing to rolling convulsions, and an occasional tonic-clonic convulsion. Death occurred from 12 min to 2 h after intravenous dosing and from 3½ to 28 h after oral dosing. Survivors were usually almost normal within 4 to 6 h after intravenous dosage, but recovery from an oral dose took up to 48 h.

The impression gained from observing the animals was that the site of action of NRDC 156 and 161 was mainly central, with little or none of the peripheral component that has been demonstrated for other pyrethroids.

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Received December 28, 1973.

<sup>1</sup> Elliott, M., Farnham, A. W., Janes, N. F., Needham, P. H., and Pulman, D. A., *Nature*, **248**, 710.

<sup>2</sup> Verschoyle, R. D., and Barnes, J. M., *Pestic. Biochem. Physiol.*, **2**, 208 (1972).

## First frog fossils from Australia

RECENT speculation about the ancestral frog fauna of Australia, even embracing the Cretaceous<sup>1</sup>, has been based purely on zoogeographic deductions, for study of the origins and history of this fauna has been seriously handicapped by the complete absence of an endemic fossil record. Although it has been suggested<sup>2,3</sup> that the Lower Eocene *Indobatrachus* of the Intertrappean beds of Bombay represents the Australian leptodactylid subfamily Myobatrachinae, such a relationship is considered dubious or untenable by other authors<sup>4,5</sup>. It is therefore of interest to report the recent discovery of isolated fragments of frog bones among a rich mid-Miocene vertebrate fauna in Central Australia, so being the first frog fossils found in Australia.

The material was obtained at Tedford Quarry, V-5375, on the west side of Lake Palankarinna in the extremely arid north of the State of South Australia. The site is in the Etadunna Formation, and the biological material recovered there is termed the Ngapakaldi Fauna by Stirton, Tedford and Woodburne<sup>6</sup>.

Most of the isolated anuran bones comprise limb fragments (particularly heads of humeri and femora) which, at present, cannot be referred to families, but there is included an almost complete left ilium (Fig. 1) which is 7 mm long and remarkably well preserved. This specimen is of particular value because of all isolated anuran bones the ilium is considered to be of the greatest diagnostic value at the familial, generic and even specific level<sup>7,8</sup>. The fossil is, however, so unusual that its familial disposition is not readily resolved.

Four families of frogs are currently recognised from Australia: Hylidae, Leptodactylidae, Microhylidae and Ranidae. Nevertheless, the disjunct distribution of the Leiopelmatidae now confined to North America and New Zealand indicates that leiopelmatids may have formerly occurred in Australia. Hence it would be unwise to restrict comparative osteological studies to families now represented.

## Toxicity of new pyrethroid insecticide

THE mammalian toxicity of NRDC 156 and NRDC 161 (see accompanying paper<sup>1</sup>) was assessed in albino female, rats 10 to 12 week old.

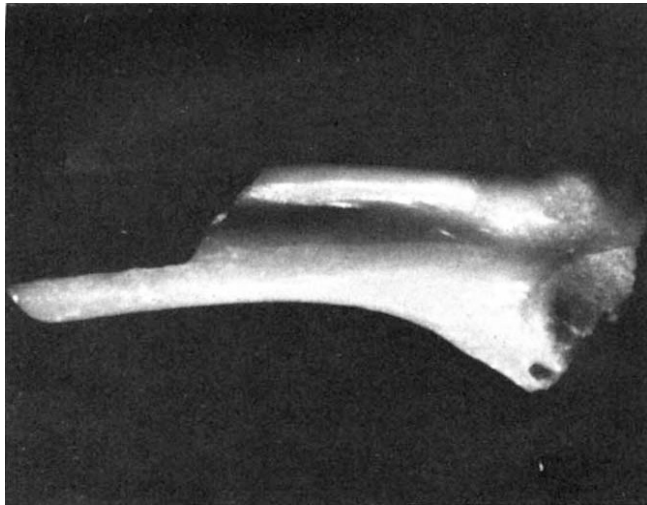


FIG. 1 Left ilium lacking only the proximal portion of the shaft and the distal tip of the dorsal acetabular expansion. (Photo: R. Wood.)

What makes the fossil so noteworthy is the presence of a deep lateral groove extending on the length of the ilial shaft. Modifications to the shaft certainly occur in several modern families, but such modifications inevitably involve the development of ridges or crests extending above the body of the shaft. In many ranids the crests are particularly well developed.

The moderate development and lateral disposition of the dorsal prominence is consistent with Lynch's<sup>3</sup> observations on Australian leptodactylids, but the acute angle at which the ventral acetabular expansion subtends to the ilial shaft is contrary to Lynch's definition of 90°–110° and my own observations (90°–130°). Provisional studies, however, favour the assumption that the fossil will ultimately prove to be a leptodactylid or possibly a hylid. Trueb<sup>9</sup>, the most recent contributor to revisionary studies of the anuran ilium has already summarised the criteria that support this contention, namely that primitive anurans "have a plain shaft that tends to be cylindrical in cross section". It has been argued by Tyler<sup>10</sup> that the Microhylidae and Ranidae probably colonised Australia no earlier than the Pleistocene. Bearing in mind the duration of the isolation of Australia, it seems likely therefore that the Miocene fauna was composed solely of hylids and leptodactylids, and possibly a perpetuated remnant of the ancestral stock from which these Australian frogs are derived.

That the Ngapakaldi Fauna is only of mid-Miocene age relies upon the palynological evidence of W. K. Harris (personal communication), for Stirton, Tedford and Woodburne<sup>6</sup> consider it late Oligocene or early Miocene. The environment occupied by the Ngapakaldi Fauna, as indicated by its content, was certainly less inimicable to anurans than the current aridity, and it follows that it is reasonable to anticipate little similarity between the Ngapakaldi anuran Fauna and the arid-adapted fauna now occurring in the extreme north of South Australia.

I thank Dr M. O. Woodburne for the opportunity to examine this material. Description awaits the completion of more detailed comparative studies.

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Received December 27, 1973.

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## Erratum

Figure 3 of the article "Aftershocks caused by the Novaya Zemlya explosion on October 27, 1973" by Hans Israelson, Ragnar Slunga and Ola Dahlman (*Nature*, **247**, 450; 1974) and Figure 3 of the article "Coral growth related to resuspension of bottom sediments" by Richard E. Dodge, Robert C. Aller and John Thomson (*Nature*, **247**, 574; 1974) were interchanged. Figure 3 of the article by Israelson *et al.* should have been:

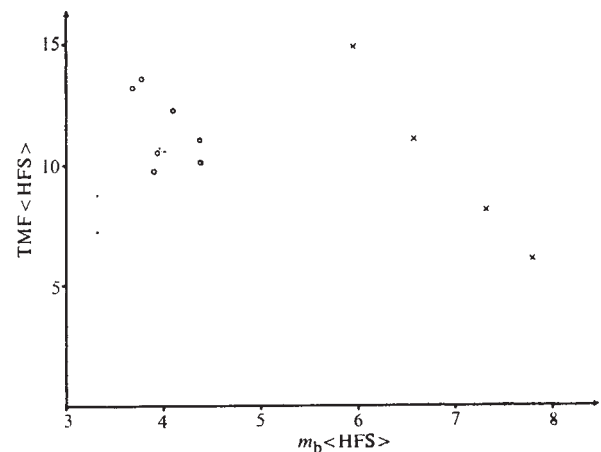


FIG. 3 Third moment of frequency, TMF, values as a function of body wave magnitude,  $m_b$  (HFS), for explosions (x) and aftershocks (o).

and Figure 3 of the article by Dodge *et al.* should have been:

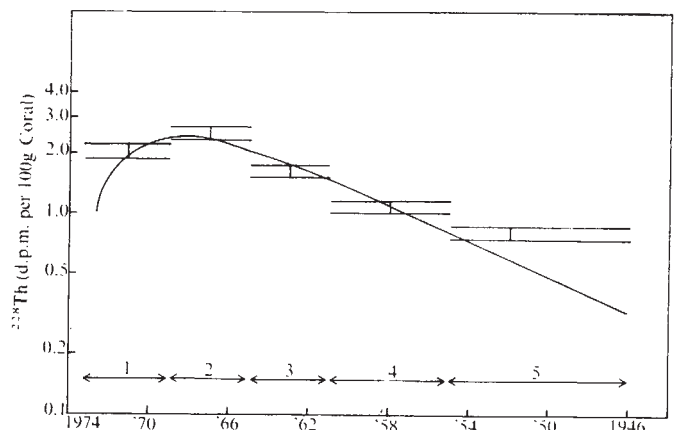


FIG. 3  $^{228}\text{Th}$  specific activities obtained for skeletal intervals shown in Fig. 2. The curve is the predicted  $^{228}\text{Th}$  ingrowth for an initial  $^{228}\text{Ra}$  value of 4.0 d.p.m. per 100 g coral.



# book reviews

## Chemistry to build reactors

*The Chemistry of Fusion Technology.* Edited by Dieter M. Gruen. Pp. xiv+394. (Proceedings of an American Chemical Society Symposium held in Boston, Mass., in April 1972.) (Plenum: New York and London, 1972.) \$22.50.

THE possibility of creating conditions in which the nuclear energy of light elements might be released in controlled nuclear fusion reactions emerged from the advances in nuclear physics in the period 1930–50. To bring these ideas to fruition, it has been necessary first to establish a major new branch of physics, high temperature plasma physics; second, this new knowledge has to be applied to potentially practical systems; and third, the technology of such systems has to be established.

With the growing progress in plasma physics and in its application to the magnetic confinement of high temperature plasmas (a turning point was the publication in *Nature* of the results of the joint Soviet–United Kingdom experiments on the Russian Tokamak magnetic confinement device in 1969) and with a growing awareness of the acute nature of the potential energy shortage, much more interest is being shown worldwide in the third area—the technology of future fusion reactors. Since very large resources have been accumulated over the years in nuclear fission technology, there is no shortage of experienced and resourceful researchers able and anxious to tackle the new technological challenge: and it is now possible for the energetic student to attend perhaps half a dozen symposia a year in which the problems of the technology of fusion reactors are scanned and discussed. Moreover, large sums of money are forecast to be available, particularly in the United States and Germany, to tackle what in 1946 George Gamow described as a problem of almost unsurpassable technical difficulty.

The present volume is a record—and a good one—of one of the most constructive of such symposia. It discusses, at a foundation level, the problems of chemical engineering which will have to be dealt with if the current physical idea proves correct: that controlled fusion reactors will be fuelled by deuterium and lithium and based on thermonuclear reactions between deuterium and tritium in an isolated high temperature

plasma. No particular system of plasma confinement or control is envisaged, so that the meat of the volume is of value whichever of the current systems advocated on physics or engineering grounds turns out to be the most practicable.

J. D. Lee of the University of California is a leading authority on the breeding of tritium in the lithium-bearing blankets, and his article (40 pages) on tritium breeding and direct energy conversion summarises authoritatively the main results of his group on neutronics of the blanket, together with short discussions of the chemical and electrochemical problems of using liquid lithium metal as a coolant in a high magnetic field. The section on direct conversion is, disappointingly, confined to a secondhand presentation of one particular system—the direct conversion of the mirror energy leakage, by R. F. Post. The review of the chemical, physical and thermal properties of lithium (70 pages, 111 references) by Cairns, Caffasso and Maroni of the Argonne National Laboratory, is a fundamental and authoritative collation of data particularly valuable because of critical assessment of the data and the extensive bibliography; it includes data on topics ranging from natural abundance, thermodynamics and physical properties to corrosion rates of selected high temperature materials in lithium. Likewise, W. R. Grimes and S. Cantor of Oak Ridge on the use of molten lithium-bearing salts as coolant and breeder (28 pages, 36 references) cover important data on one of the major alternative constituents of the blanket, and their article is based on Oak Ridge experience of the Molten Salt Reactor. E. F. Johnson of Princeton—another leading authority—deals (22 pages, 21 references) with the chemical problems of tritium extraction from the blanket, but rather too briefly and qualitatively to be of lasting value. His article does, however, give a useful summary of the Princeton reference design model reactor which provides the non-specialist reader with a concrete example of present-day reactor concepts.

D. M. Gruen provides a useful and interesting account (24 pages, 57 references) of the often-neglected chemical effect of plasma interaction with thermonuclear reactor surfaces, together with a brief review of more familiar surface topics. R. E. Stickney provides a major

and critical account (78 pages, 110 references) of the permeation of hydrogen isotopes through high temperature materials—a matter of great importance to the assessment of the spread of tritium around and through the components of envisaged reactors. Finally, the chemical content of the volume is completed by a short (16 pages, 31 references) article by G. G. Libowitz on condensed-metal hydrogen systems, and a rather superficial article on superconducting materials.

To the fusion reactor specialist, the main value of the book rests in the bringing together of today's main chemical knowledge relevant to fusion reactors, or at least fusion reactor blanket systems. To the chemist, the book indicates the main areas of chemical technological interest provoked by current fusion reactor concepts. The impression for the non-specialist is likely to be that each individual problem of chemical technology so far raised and discussed has outline solutions, but the sum total of the problems constitutes a serious technological barrier which—as in the case of fission reactors—can seem daunting in the initial stages. There is clearly a great need to improve much of the chemical data and understanding, particularly in the difficult areas of corrosion (where the effects of trace elements are not understood), permeation and radiation chemistry.

R. S. PEASE

## Solid electrolytes

*Physics of Electrolytes.* Edited by J. Hladik. (Transport Processes in Solid Electrolytes, and In Electrodes vol. 1. Pp. xiii+516. (Academic: New York and London, August 1972.) £11.00; \$34.

THIS series of review articles was presumably intended to provide the background knowledge required for investigating the properties of ionically conducting solids, a field which is currently attracting considerable interest. The topics covered in this volume are grouped into two sections; "Solid Electrolytes and Electrodes" and "Transport Processes".

The first section contains a rather heterogeneous collection of articles. In the first two chapters, Hladik discusses electronic theories of the solid state, interatomic distances, the cohesive energy in ionic crystals and also gives a gen-

eral account of solid electrolytes. Unfortunately, the relevance of many of these topics to the study of solid electrolytes is not made clear. The discussion of interatomic distances is rather inconclusive and that on cohesive energy suffers from a lack of references to the tabulated data; this is particularly irritating when the data in two tables (X and XII) do not agree.

Fong has contributed an account of the statistical mechanical treatment of interactions between impurity atoms and lattice defects in ionic crystals, and the experimental results available to test this theory. In the final chapter of this section, Amsel describes the range of applicability of, and some of the experimental results obtained with nuclear microanalysis. This chapter makes extremely interesting reading but deals mainly with film formation on metal surfaces.

The section on transport processes is much more homogeneous. Friauf gives a sound account of the basic theory of conduction and diffusion in solids and describes the methods available for determining the mechanism of these processes. Diffusion processes are also clearly discussed, though in more detail, by B  ni  re but there is considerable overlap between these two chapters; some of the tables and figures and much of the theory being common to both. B  ni  re has also contributed a good but incomplete account of transference number measurements in ionic crystals. The measurement of the transference number of the electron by EMF measurements or by the Wagner technique is unfortunately not described. Ionic conductivity in solids is reviewed by Kvist but only a cursory account of the experimental methods and the conditions which must be satisfied in making conductivity measurements is provided.

The remaining chapters by Hartman, on ionic conductivity in whiskers, Hughes and Isard, on ionic transport in glasses, and Riande, on transport phenomena in ion-exchange resins, are very competent reviews.

Overall, this book suffers from inadequate editing to remove the unnecessary overlap between some chapters and to provide the links between the separate contributions. The proof reading, especially of the chapters by Hladik, must have been careless and should have ensured that the tables and diagrams in the chapter by Kvist appeared nearer to the relevant sections of the text.

In spite of the limitations described above, this collection of articles and especially those on transport phenomena, should be of value to those involved in this area of research.

T. DICKINSON

## Copernicana

*The Scientific World of Copernicus: On the Occasion of the 500th Anniversary of His Birth, 1473-1973.* Edited by B. Bienkowska. Pp. xii+142. (Reidel: Dordrecht and Boston, 1973.) Dfl. 50.

1973, half a millennium since the birth of Nicholas Copernicus the founder of modern astronomy, has produced an unprecedented outpouring of Copernicana. To the present example twelve authors contributed essays, three previously published in Polish and one in French.

Discrepancies between statements by different contributors have been overlooked by the editor. Thus Copernicus is said to have spent "five years as [a] student in Cracow" (page 1). Yet "Copernicus enrolled at the University of Cracow in 1491. For the four years he remained at the University he devoted himself to the study of the liberal arts without, however, winning an academic degree" (page 15). Was Copernicus at Cracow four years or five? The university records do not reveal when he left. At Cracow the bachelor's degree was normally obtained in four years. Surely the founder of modern astronomy was no dullard. Since Copernicus received no degree from Cracow, and did not need the baccalaureate for the career which he contemplated, presumably he stayed less than four full academic years in Cracow.

Readers are assured that the thesis of Copernicus' *Revolutions* "was accepted . . . without demur by Pope Paul III, to whom Copernicus' book was dedicated" (page xi). For this familiar assertion about the reigning pope, nobody has ever adduced any documentary support. Evidence to the contrary was published in *Rivista critica di storia della filosofia* (26, 84-85; 1971). Shortly after Copernicus' *Revolutions* was printed in 1543, initiating modern astronomy and therewith modern science, preparations to condemn that magnificent work were begun by Pope Paul III's personal theological advisor, Bartolomeo Spina, the Master of the Sacred and Apostolic Palace. But before Spina could act, he fell ill and died. Thereupon Spina's close friend Giovanni Maria Tolosani resumed the theological anti-Copernican campaign in an appendix to his treatise *On the Truth of the Holy Scripture*. Herein Tolosani declared that Copernicus "contradicts some Scriptural principles, not without danger of infidelity both to himself and to the readers of his book". An annotation in Tolosani's manuscript discloses that his attack on Copernicus "for the purpose of safeguarding the truth to the common advantage of Holy Church" was later consulted by the preacher of

the first public sermon against that great Copernican, Galileo.

The (lost) reconciliation of Copernicanism with the Bible was written by Copernicus' disciple Rheticus, not by Bishop Giese (page 27). The "first publisher" of Copernicus' *Revolutions* was Petreius, not Osiander (page 140).

EDWARD ROSEN

## Chromosome more or less

*Cytogenetics of Aneuploids.* By Gurdev S. Khush. Pp. xii+301. (Academic; New York and London, December 1973) \$17.50; £8.40.

THIS book is a survey of what has been achieved in isolating, identifying and using aneuploids, that is, individuals whose chromosome number differs from an exact multiple of the basic number. Most of the book is concerned with aneuploids in plants, with a very short chapter in which the author briefly describes examples of aneuploids in animals, especially man.

After outlining concisely the history of the discovery and production of the various aneuploids, and the terminology used for the different types, the author gives a very useful glossary of the current terms, and an explanation of the chromosomal formulae used to indicate the different types of aneuploids.

The book is then divided into two main parts, the first dealing with trisomics, with some mention of tetrasomics, and the second dealing mainly with monosomics but also mentioning nullisomics. As monosomics and trisomics have seldom been developed in the same species, this does not lead to too much duplication, and the two sections are linked by cross references where necessary. Each part covers the sources of the relevant aneuploids, detailing how the various types have been obtained in different species; some of the difficulties encountered in producing the aneuploids; the cytological behaviour associated with the aneuploid condition; and the breeding behaviour of the aneuploids. There are chapters on the uses of aneuploids, which include chromosome mapping, locating markers on chromosomes, locating the centromere and orientating the linkage map on the chromosome, as well as the production of substitution and addition lines.

The book brings together data widely scattered in the biological literature, and so will be a valuable reference work for those wishing to develop or use an aneuploid series, as well as being a useful source of information for anyone requiring a basic knowledge of the subject. It will therefore be of use to honours undergraduates as well as practising cytogeneticists and plant breeders.

J. P. MOSS



## Full attention

*Attention and Effort.* By Daniel Kahneman. Pp. x+246. (Series in Experimental Psychology.) (Prentice-Hall: Englewood Cliffs, N.J., July 1973.) \$8.95.

BETWEEN the wars the topic of attention was neglected by psychologists, in their enthusiasm for predicting each response as a function of the immediately previous stimulus. The difficulty was, however, that the effects of a stimulus depend upon the state in which a man is when he receives it. Sometimes he reacts to it, will say verbally that he has noticed it, will remember it later; and sometimes none of these things will happen. The processes which can be variously and roughly described as gating, filtering, or selecting among inputs to the system have therefore become a live area of research: and books on attention are now almost too numerous. They range from the massive, detailed, and unreadable to the popular, chatty and superficial. The competition for another is tough.

It is a pleasure therefore to be able to declare this book the best one on the subject thus far. It is short, clear, and extremely up to date: not merely are there references to publications in 1973, but also exciting accounts of new experiments by the author and his students in Jerusalem, which will be carefully read by advanced workers in the field. At the same time, the coverage of the literature is wider than in most of the competitors, and the author understands sympathetically what is being argued by other investigators. Thus for once this is a book which can be recommended, not only to the advanced workers already mentioned, but for teaching purposes, and even conceivably for non-psychologists who wish to find out the latest state of the art in this area.

For those who are already expert, one can indicate the position the book adopts by saying that it takes broadly the position of Treisman, but with much greater emphasis and experimental detail on Neisser's pre-attentive processes, and on recursive influences from processes at one stage of recognition upon others both earlier and later in the system. The findings of the past two or three years make it possible to do this convincingly and briefly, and Fig. 5-1 is perhaps the first diagrammatic representation of attention which improves upon that of Treisman in 1960.

Criticisms? The book is weaker when it turns to motor performance rather than perception, which is reasonable because the state of knowledge is less satisfactory: and there are perfectly adequate references to Posner, to Keele,

to Brown and to Trumbo and Noble for those who wish to go further. Those of us who work on the effects of noise and of other stresses will be disappointed by the way in which effort, arousal, the allocation of resources and the process which determines the size of the pupil are all treated almost interchangeably. Perhaps one can most fairly put it this way: there are real differences, which experimental evidence compels us to draw, between easy and involuntary attention such as that with which we follow the graceful prose of this author; and the determined concentration, sustained by a hope of remote goals, with which we mobilise all our resources to follow the confused thinking of some others. These differences are not yet understood, but for what we know now, Kahneman is the best guide.

DONALD F. BROADBENT

## Nematode interactions

*Nematode Ecology and Plant Disease.* By H. R. Wallace. Pp. ix+228. (Arnold: London, December 1973.) £6.50.

THIS is a readable and interesting account of nematode ecology and the role of nematodes in plant diseases. Early chapters discuss nematode injury to plants, plant growth in relation to numbers of nematodes and plant responses to nematode invasion and feeding. The second half of the book deals with distribution, abundance and adaptations of plant-parasitic nematodes in the environment and with their involvement in specific diseases. The final chapter suggests how the role of nematodes in plant diseases should be investigated.

Unlike Dr Wallace's earlier book (*Biology of plant-parasitic nematodes*; 1963) this is no textbook, for it is far from comprehensive and the references are nearly all drawn from the last decade. The text is often speculative, not a bad thing, but it is also at times superficial, and with some conclusions that are debatable. The book is best where, as in parts of the chapters on nematode ecology, it deals concisely with its subject. Too much of the text, in my opinion, is taken up with fulsome descriptions, definitions and debates on abstractions such as 'health', 'disease', 'homeostasis', and ecological jargon and with the views of medical and plant pathologists.

Too little attention is devoted to well documented evidence of the relationship of nematodes with plant diseases. For example, much is known of nematode-fungus interactions in plant disease complexes and of nematodes as vectors of plant viruses, both of which receive scant attention. The inferences drawn about the work on Docking disorder are incorrect. Nor does Wallace indicate

how much can be learnt from careful study of plant symptoms and from inoculation experiments with suspected pathogens.

Instead, he stresses the use of multifactorial analyses of all the environmental variables that can be thought of and measured, to indicate which factor or combination of factors is most likely responsible for the particular disease. To my mind this is putting cart before horse. Not all diseases involving nematodes are as complex as Dr Wallace implies or we would be without commercially successful cultivars bred for resistance to specific nematodes. Despite such criticisms I commend this book to all thoughtful nematologists, plant pathologists and ecologists.

A. G. WHITEHEAD

## Physicists talking

*Fundamental Interactions in Physics: 1973 Conference at the Centre for Mathematical Physics in the University of Miami.* Edited by Arnold Perlmutter. Pp. ix+399. (Studies in Natural Sciences vol. 2.) (Plenum: London and New York, 1973.) \$29.

A CONFERENCE on so wide a subject as this one is in danger of losing coherence and producing a set of papers from so great a range of disciplines as to require from any one scientist an enormous effort of comprehension, and yet to include contributions so speculative as to make such effort unworthwhile. This familiar danger is avoided by the present volume, largely because of the elegance and informality of the presentation of the papers which seem to have been presented as talks and recorded verbatim. Here, perhaps, is a solution to the problem of scientific style on which John Maddox commented in a recent BBC radio broadcast: "... when a scientist sits down in front of his IBM typewriter. . ."

There are three sections in the book. The first deals with particle physics and is largely theoretical though it includes an informal description of work at the National Accelerator Laboratory (Batavia, Illinois) and a paper by Hofstadter on applications of absorption detectors. The theoretical papers range from a quasi-classical discussion by P. A. M. Dirac of symmetry breaking in a new variation of Weyl's theory of the electromagnetic field, to a terse and wholly algebraic "Approach to Hadron Physics . . ." by Oneda and Matsuda. Several of these papers contain much that is new, and some of them contain fertile ideas rather than completed theories. For example, Bopp and Lutzenberger in a short contribution throw out a simple model of quantised retarded forces in which the electron and the muon appear as eigensolutions sub-



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ject to the correctness of guesses about the bare masses and angular momenta of the particle in their model. But at least three of the papers (by Kurusunoglu, Salam and Blokhinstev) have a more didactic purpose and will be of great use to graduate students of particle physics.

The papers in the second section on atomic and molecular physics, one by Massey, one by Spruch and one by Herzenberg, are nearer to reviews than those in the first, but there are also two panel discussions. Three more speculative papers on X-ray astronomy, helium and biology make up the third section.

Despite the relatively high speed of the book's production the presentation is excellent and free of misprints. Thus a scattered but uniformly high powered, original but well written collection of papers make up a book which particle physicists will find stimulating and informative. I hope that university libraries (to whom the high price presumably restricts the book's sale) will make it available to them.

PHILIP ROE

## Thinking protozoa

*The Evolutionary Foundations of Psychology: a Unified Theory.* By Felix E. Goodson. Pp. xi+228. (Holt, Rinehart and Winston: New York and London, 1973) n.p.

DR Goodson sets himself the Kantian goal (the parallel is mine) of effecting a transcendental deduction of the psychological factors necessarily involved if the evolution of complex organisms is to be possible. In other words, he aims for an integrated view of psychology derived by way of abstract arguments from a broadly evolutionary base.

Unfortunately, although he has some interesting things to say about the psychological necessities inherent in the earliest forms of life, his concepts are not sufficiently structured or precise to add much to one's understanding of mammalian and human psychology. To be sure, his stress on adaptive function for the sake of the organism as a whole is preferable to an excessively narrow conception of psychology that ignores man's evolutionary context. And he relates a wide variety of findings in empirical psychology to this overall perspective. But his remarks are too vague to be helpful, and leave most interesting substantive questions unanswered—or, worse, unasked.

For example, he identifies the paramount task of psychology as "an evaluation of apperception that focusses on the variables that affect the rapid shifting from one component to the next", where by "apperception" he means the process (often called "attention") by which inputs from the senses or the

memory come briefly into focus in the conscious mind (page 68). Whether or not accounting for the detailed course of the stream of consciousness is 'the' paramount task of psychology, it certainly is an important psychological problem. But what does Dr Goodson have to say on the subject?

"Thinking", he says, "is nothing more than fluctuation of apperception among available encodes and as such is a completely automatic and lawful process" (page 135). Readers of *Nature*, who are not prone to think of thinking as a random activity, can hardly demur with this—but what one wants is some indication of the nature of the laws involved. Precise specification, of course, is not to be looked for in a deliberately general and abstract discussion. But one might reasonably hope for a more powerful concept than 'equilibration' (which is involved in Goodson's most basic "evolutionary postulates of process") an indication of the psychological complexity of thought. Thus the characterisation of "thinking" quoted above continues: "Apperception is always the most equilibratory response possible to the myriad components dynamically interacting within the integration matrix, and when the components brought into sequential focus consist of encodes, thought is taking place".

If a generic concept of equilibration is to be applied equally to the specifics of chemical diffusion in protozoa and flights of fancy in human beings, more needs to be said about the differential aspects of the phenomena so confidently classified together. Even the social psychological theories of "cognitive consistency" and "cognitive balance", vague and unsatisfactory as they may be, make a better attempt than Goodson to state what constitutes "equilibration" in differing types of thought. Equally, Freud offered more substantive suggestions about the structure of the "integration matrix" and the varieties of "psychic disequilibrium" than can be found in this book. It is ironic that Goodson castigates psychoanalytic theory for "its reliance on vague terminology" (page 204).

I should perhaps admit my own conviction that the complexities of mental structure can only be satisfactorily approached by way of the concepts currently developing within artificial intelligence. My dissatisfaction with this book doubtless reflects the measure of its distance from this theoretical approach. But I think it unlikely that psychologists favouring other paradigms would find it a rich source of insights. Even Kant tried to show in detail how specific examples of human thinking (such as the metaphysical antinomies) were generated by the abstract forms of thought postulated by his transcendental deductions. MARGARET A. BODEN



# science on radio

## Bush House bonanza

John Gribbin and Eleanor Lawrence

One of the strangest economies proposed in the latest round of British belt-tightening is to cut back drastically on the funds available to the external services of the BBC, which operate from Bush House, London. These services have already suffered a small decline in the amount of cash available to them—which represents a significant decrease in real terms, after inflation has done its worst. Yet the BBC continues to be held in the highest esteem by listeners around the world, who find its services informative and, relatively, free from propaganda. Of course, even the BBC is not completely unbiased: its science, industry and agriculture unit, for example, makes no bones about the fact that although it hopes to report fundamental developments in pure science wherever they are made, technological applications with commercial applications are generally only 'promoted' if they are British. But that understandable bias is hardly in the same league as the political propaganda of the other giants of world broadcasting.

Compared with the domestic services of the BBC (and, indeed, the internal broadcasting services of other countries) Bush House provides a veritable scientific bonanza. Out of 168 hours of broadcasting in English each week, there are two half-hour programmes devoted entirely to science (each of them repeated twice), a nature notebook and a programme for farmers. With more or less regular scientific features (12 to 20 a year, each running for 30 minutes) and the tit-bits of science found in news and current affairs programmes, the total is something like 5 percent of the weekly output.

This is achieved with a very restricted budget, roughly half of the amount available for comparable domestic programmes (such as the late, lamented "Kaleidoscope"); if the funds of the science unit are cut back at all there seems no way in which economies could be made except by reducing the number of programmes. It may not happen, and it certainly should not happen, to judge from the quality, as well as the quantity, of these programmes.

Of the two main science unit programmes, "Science in Action" aims "to inform and sometimes amuse" the non-

specialist listener. We were a little surprised by the high scientific standard of this programme—but of course it is broadcast in English, and therefore its audience overseas must be better educated and more well-informed than the average population. Audience response and a recent survey indicate that medical items are most popular, with communications and astronomy following some way behind. Questions asked by listeners indicate how successfully the programme communicates with its intended audience: would two children brought up together in isolation from birth learn to communicate and what are hypertension and vertigo?

At a high level, "Discovery" aims to be by scientists and for scientists, and beats anything else we have heard on domestic radio or elsewhere hollow. In the half-hour programme, two or occasionally three scientists are interviewed about their own work and given free reign. The result is something like a radio version of the "News and Views" section of *Nature*.

In one programme we heard, Dr V. Marks from the University of Surrey talked about gut hormones and insulin control, and Dr Jeffrey Manning from the Rutherford Laboratory explained their current accelerator research programme. This did not duck what seemed to us the contentious question—especially to an audience in the developing countries—of whether an accelerator costing £15 million is justifiable. But it seems that there is, in fact, a more general recognition of the value of fundamental research in the countries which can least afford it themselves.

This raises another interesting point: the lively postbag received by the science unit includes contributions from the poorest countries, where the cost of posting a letter to Britain can be as much as 50 percent of a week's earnings, yet many listeners feel it worth the cost of responding in this way. One correspondent, an Indian doctor, said how much he valued the programmes as his only effective means of keeping up to date, since the cost of "the literature" is quite prohibitive for him.

Part of this listener interest must stem from the immediacy of the programmes. Special features, such as one discussing the causes and implications of the present droughts in sub-Saharan Africa, can be put together within 72

hours, and in a "Science in Action" programme broadcast in the week ending April 6 Dr Simon Mitton, of the University of Cambridge, could be heard giving details of the Mariner-10 observations of Mercury which were certainly new to us. This immediacy is aided, of course, by the happy relationship between Bush House and active scientists, who are, it seems, often pleasantly surprised at the difference in approach between the external services and the domestic TV services.

As well as the programmes broadcast from London, the BBC also provides a service for radio stations around the world in the form of taped programmes. And this really is a service—a weekly fifteen minute tape programme, for use intact or to be cut up and inserted into other programmes, costs only £1.50 plus postage, and there is no obligation for the user even to credit the BBC when an item is used. One of these tapes, "Science Magazine", has a link with *Nature* in the form of a weekly contribution from one or more members of the editorial staff: this programme is a mixture of short items and a long interview, almost a combination of "Science in Action" and "Discovery". It has not always come off as a programme in itself, although at the giveaway price excerpts find outlets around the world, not just in developing countries but also on American public and campus radio. We have, however, heard a pilot of a 'new look' "Science Magazine", with more punch and a lively signature tune: this format seems more likely to provide a good listen and makes more sense for users who wish to broadcast the programme intact.

All in all, the science output from Bush House is difficult to fault. The fundamental reason for this seems to us to be that the producers themselves and most of the presenters all have scientific training, although the audience is not entirely science oriented, as correspondence from insomniacs and long distance lorry drivers in Britain indicates. Far from reducing this valuable output, the powers that be should be encouraged to promote it as a valuable string to the BBC's bow, in the best traditions of Reith. Perhaps they could even take note of the most common plea from those insomniacs and list the programmes, in as small a print as they like, in the *Radio Times*.

# instrument review

## Surround sound

John Wilson

IN an attempt to capture both the natural direction and reverberation of sound in a live performance, Professor Peter Felgett of the University of Reading and Michael Gerzon, an Oxford mathematician, are developing a new 'ambisonic' hifi system in association with IMF, a British loudspeaker manufacturer. The National Research Development Corporation holds the patents for the system and is backing the project financially.

The new technique improves on present quadrasonic systems because of its ability to present natural sound images between the front and rear pairs of speakers, and to reproduce sounds which seem to arise either between the listener and a loudspeaker or beyond it. Indeed Mr Gerzon believes that: "Quadrasonics, as at present widely conceived, is a dead end."

Unlike the conventional quadrasonic approach, where a separate channel is so often deemed necessary for each loudspeaker, the new ambisonic system uses information from a multidirectional microphone array encoded on just two channels. This should lead to the complexities and sophistication of surround sound techniques being relegated to the recording studio and not to the living room. Indeed, apart from two extra loudspeakers, suitably in phase, only a decoder will be necessary to convert an ordinary stereo system.

This new approach should not be confused with the so-called 'matrix'

systems where information from conventional microphones is artificially blended to achieve an approximation of surround sound. With ambisonics, sound from every direction reaching the tetrahedral microphone array is treated equally until the decoding operation.

Michael Gerzon, who Professor Felgett feels should take most of the credit, points out that an ambisonic system is not limited to four loudspeakers, although this is the number which is most attractive commercially. Since the microphones also pick up the sounds coming from above and below them, these too may be reproduced if six or more loudspeakers are used with the appropriate decoder. Three is the optimum number of channels for four speakers (and therefore fm radio transmission), five channels for six loudspeakers, six for seven and so on. The position of the loudspeakers must be very carefully worked out because they are not in the usual planar arrangement.

*Nature* hopes to publish an article describing the mathematics behind the new system in the fairly near future; but how does it sound in practice? The new system was demonstrated at the recent Sonex 74 exhibition in the Post House Hotel near London's Heathrow airport. Four expensive monitor speakers were supplied for the occasion by IMF but the signals themselves came from an ordinary Philips cassette.

Just before the demonstration began, a director of IMF, Mr John Wright, said a few words of warning about the acoustics in the demonstration room. He should have said more because the organisers could hardly have picked a

worse spot. One wall of polished mahogany bounced the sound like a mirror through an acoustically transparent partition opposite and even the other, more normal, wall was faced by a large window with heavy, absorbent curtains. So the demonstration was rather disappointing, particularly regarding the claim that the nearer a listener is to particular speaker, the less sound he is conscious of hearing from it. This simply could not be true in a room where the sound was escaping next door through one wall and being smothered in the curtains of another.

In spite of this one or two of the selections on the tape gave some idea of what an ambisonic system is capable. In particular, a fine piece of organ music produced the strong impression of being in an echoing church. Considering the real surroundings, this was no mean feat and Professor Felgett said that he hopes to arrange another demonstration in a more suitable location.

The technique is still very much in the experimental stage, with new directional microphones being developed, for example, and the inventors emphasise that they have no plans yet to market it themselves. Rather, they wish to attract the attention of recording studios and manufacturers with a view to licensing their invention—much in the same way as Dolby has licensed his method for 'stretching out' the hiss from tape recordings. In this respect Professor Felgett says that IMF is acting like "the good farmer" by seeking to develop the system for the benefit of all, although it naturally has a commercial interest in being in the forefront of a new technique.

# matters arising

## The information problem

SIR,—K. L. and M. L. Blaxter, in their article *The individual and the information problem* (*Nature*, 246, 335) make some incidental remarks which cannot pass unchallenged. They say, for example, that the "so-called information explosion . . . apparently causes consternation to librarians, archivists and information scientists". No evidence is quoted

in support of this suggestion, perhaps because it would be difficult to come by. Whatever consternation exists is, in fact, more likely to be found among the ranks of the users than of those of us professionally engaged in dealing with the problem. We are far too busy actually harnessing the forces of the information explosion to have time to strike attitudes of consternation.

Bryan<sup>1</sup> summed up the viewpoint of

many librarians when he argued that the information problem is essentially a users problem, and not primarily a technical problem. The remark is also unfortunate in that it seems to imply an inactivity in the face of crisis; a glance at, for example, the current issue of *Library and Information Science Abstracts* may help to dispel this misconception. Most of the Blaxters' findings are already well known to librarians, and we need no convincing



as to the desirability of such things as the application of objective criteria to the purchase of periodicals. At least one university library, Surrey, is now basing its periodicals purchasing policy on studies of use<sup>2</sup>, and the possible use of such aids as the Institute of Scientific Information's *Journal Citation Reports* is being actively examined in other libraries. Secondary journal citation is another approach being studied<sup>3</sup>.

It is also suggested that for a scientist to admit that he could not keep abreast of his own speciality would imply an incompetence. There is a confusion here between two separate and distinct areas of competence; competence as a scientist in a particular field of science, and competence as an information searcher. These two abilities are different, and the first does not necessarily imply the second. Many writers have commented on the difficulties encountered by scientists in using the literature<sup>4-8</sup>, and some scientists are prepared to admit their limitations in this field<sup>9</sup>. If past work is to be fully utilised, and duplication avoided, it is essential for all scientists to be aware of their own personal limitations, and to seek whatever assistance they require from professional information workers.

As long as there remain scientists who regard it as somehow *infra dig* to ask for advice or help on information problems, then some at least of these problems will be of their own making.

Yours faithfully,  
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<sup>1</sup> Bryan, H., *Aust. Lib. J.*, **17**, 389 (1968).

<sup>2</sup> *Annual Report, 1972-1973* (University of Surrey Library, 1973).

<sup>3</sup> Windsor, D. A., *Spec. Libr.*, **64**, 446 (1973).

<sup>4</sup> Urquhart, D. J., in *University Grants Committee, Report of the Committee on Libraries*, 280 (HMSO, London, 1967).

<sup>5</sup> Urquhart, D. J., *Aslib Proc.*, **18**, 351 (1966).

<sup>6</sup> Wood, D. N., and Barr, K. P., *J. Doc.*, **22**, 22 (1966).

<sup>7</sup> Bottle, R. T., in *Progress in Library Science 1967* (edit. by Collison, R. L.), 109 (Butterworths, London, 1967).

<sup>8</sup> Line, M. B., *J. Libr.*, **1**, 211 (1969).

<sup>9</sup> Schur, H., and Saunders, W. L., *Education and Training for Scientific and Technological Library and Information Work*, 37 (HMSO, London, 1968).

## The individual and the information problem

SIR,—Blaxter and Blaxter raise a number of interesting points in their recent paper (*Nature*, **246**, 335; 1973). They are careful to emphasise that their results and conclusions obtain from the views and habits of full-time research

scientists working in three biological research institutes, and that these may well differ from those of university lecturing staff, with educational commitments in addition to those of research. I should like to comment here on just one conclusion from the Aberdeen survey, however, and suggest that it may indeed be more generally applicable.

The Blaxters were able to estimate a figure for the total number of journals to be taken by a research institute's library to give 90% satisfaction to 90% of its users, a figure which was 25 times the number of 'fields' covered by the institute. The concept of a 'field' was defined by the publishing patterns of the individual scientist and the whole institute. For instance, for the institute cited, the number of non-overlapping fields was calculated to be five, so that the theoretically optimal number of journals to be taken by the library was only 125.

In 1971 a survey was conducted in Edinburgh by interviewing a representative proportion of the staff of the medical school with the main purpose of obtaining an estimate of the journals that our central medical library ought to take to give reasonable satisfaction to its readership. Our survey was designed rather differently from the Aberdeen one and full details of its methodology and general results have been published<sup>1</sup>. From the results, we were able to obtain a total figure of approximately 580 unique primary and review journals covering all the major subject areas of biomedicine, except for social medicine, public health and dentistry, which were outside the scope of our survey. I must admit that we were pleasantly surprised and relieved that the figure was not higher.

For the library of a research institute, a holding of 580 carefully chosen journals is theoretically optimal for 23 non-overlapping fields with 25 journals per field, by the Blaxters' method of analysis. In the Edinburgh medical school survey, the 580 unique journals were in fact derived almost entirely from 47 subject areas as defined by the Index Medicus List of Journals. This corresponds to an average of 12 to 13 unique journals per subject area. Many individuals in our survey had an interest in the journals of more than one subject area, and between the various medical school departments there was evidence for considerable overlap of interest. It would therefore seem not unreasonable if a 'field' in the Blaxters' sense approximates roughly in size to two average subject areas in the Index Medicus sense.

Approximate techniques inevitably have to be used to estimate the optimal journal holding for a library serving a readership of any complexity. The important point is that, though based on

different methodologies, both the Aberdeen and Edinburgh surveys produced the same conclusion: in spite of the information explosion, the optimal journal holding of a research institute or medical school library appears not to be so enormous after all, given, of course, adequate back-up facilities by a national loan service. In fairness to Edinburgh's medical school and library staff, I should add that financial restrictions have prevented our central medical library from as yet achieving the estimated optimal number of journals.

Yours faithfully,

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<sup>1</sup> Whittle, E. D., *Br. J. med. Educ.*, **6**, 306, (1972).

## Cognitive Subjective

GREGORY<sup>1</sup> should have acknowledged sources in references to his experiments and discussion of what he calls "cognitive contours" or, as others would have it, "subjective contours". ("Subjective contours" represents a neutral description, whereas "cognitive contours" suggests the role of an intellectual process.) He acknowledges Kanizsa as the source for his Fig. 1, but does not do so for his Fig. 2c, a figure also to be found in Kanizsa (Fig. 13)<sup>2</sup>. Gregory reports, in regard to one of his experiments, that the subjective contours of the Kanizsa figure can be obtained stereoscopically by proper presentation of parts to each eye. I had already reported the positive results of this experiment with a slightly modified Kanizsa figure, also showing diagrammatically the selection of parts for presentation in a stereoscope (page 296, page 405 n. 14; ref. 3). In another experiment, he points out that the sides of the subjective triangle appear curved when the edges of the sectors are not collinear, an effect which he describes as "new". I had, however, also pointed this out: "The Kanizsa diagram itself can be modified so that the subjective contours are curved. The alignment of the edges of two sectors constitute a condition for straightness of subjective contours . . . When the sectors are redrawn so that the edges are not aligned, a triangular shape with curved contours is seen" (page 404; ref. 3). Furthermore, I had noted the presence of subjective contours in the after-image of an incomplete figure (page 406 n. 22; ref. 3), as had also Gregory.

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Received October 27, 1972.

- <sup>1</sup> Gregory, R. L., *Nature*, **238**, 51 (1972).
- <sup>2</sup> Kanizsa, G., *Rivista di Psicologia*, **49**, 7 (1955).
- <sup>3</sup> Pastore, N., *Selective History of Theories of Visual Perception: 1650-1950* (Oxford University Press, New York, 1971).

*Nature* regrets delay in publishing this manuscript. A part of this delay can be attributed to the transition from one Editor to another.

## Announcements

### Appointments

A. L. Armitage, Vice Chancellor of the University of Manchester, has been elected **Chairman** of the **Committee of Vice Chancellors of the United Kingdom**.

The following have been elected **Fellows** of the **Royal Society**: W. F. Bodmer; W. R. Boon; K. Burton; H. J. F. Cairns; R. Y. Calne; D. R. Curtis; J. F. Davidson; J. D. Dunitz; P. V. Edman; J. D. Eshelby; J. Halpern; S. W. Hawking; V. Heine; R. A. Hinde; A. E. Litherland; J. E. Lovelock; D. H. Matthews; R. E. F. Matthews; P. D. Mitchell; S. V. Perry; N. J. Petch; J. R. Philip; J. C. Polkinghorne; C. W. Rees; J. Rishbeth; R. V. Short; J. T. Stuart; R. H. S. Thompson; R. J. Vane; F. J. Vine; S. E. Woods; and P. H. J. Young. D. H. Wiffen has been appointed **Dean** of the **Faculty of Science** of the **University of Newcastle upon Tyne**. Sir Eric Roll has been elected as **Chancellor** of the **University of Southampton**.

### Awards

The **Royal Geographical Society's Medals and Awards** have been given to the following: C. Bonnington (Founder's Medal); G. de Q. Robin (Patron's Medal); C. A. Fisher (Victoria Medal); H. H. Lamb (Murchison Award); Captain D. W. Haslam (Back Award); M. A. J. Williams (Cuthbert Peek Award); A. R. H. Baker (Gill Memorial); Colonel J. M. Adam and I. Douglas-Hamilton (Ness Awards); and D. Bartlett and J. Bartlett (Cherry Kearton Medal and Award). The **Council of the Royal Society of Victoria** has awarded **Research Medals** for 1973 to D. Metcalf and J. H. Willis. W. T. Elwell of **Imperial Metal Industries Ltd** has been awarded the **Gold Medal** of the **Society for Analytical Chemistry**.

### Misscellaneous

The **International Health Foundation** (1 place du Port, 1204, Geneva) has an-

nounced that the theme chosen for its award scheme this year is **Oral Contraception—a 1974 critical assessment**. Applications are invited by the **Council of the Royal Society** (6 Carlton House Terrace, London SW1Y 5AG) for two **Royal Society J. Sainsbury Research Fellowships**.

### Erratum

In the obituary of C. A. Coulson (*Nature*, 248, 367 (1974), who died on January 7, 1974, it was stated that he entered Trinity College, Cambridge from his local Grammar School. In fact he was educated at Clifton College, Bristol.

### Reports and Publications

not included in the *Monthly Books Supplement*

### Great Britain and Ireland

- The Mental Health Trust and Research Fund. Annual Report and Accounts, 1973. Pp. 28. (London: The Mental Health Trust and Research Fund, 8 Wimpole Street, W1, 1973.) [1712]
- UKAEA—Research Group. AERE-R 7540: Radioactive Fallout in Air and Rain—Results to the middle of 1973. By R. S. Cambray, Miss E. M. R. Fisher, A. Parker and D. H. Peirson. Pp. iii+38. (Harwell, Berkshire: Environmental and Medical Sciences Division, Atomic Energy Research Establishment. Available from HMSO.) £1 net. [1912]
- International Computers (Holdings), Limited. Annual Report and Accounts, 1973. Pp. 24. (London: International Computers (Holdings), Ltd., 1973.) [1912]
- Provisional Geochemical Atlas of Northern Ireland. (Technical Communication No. 60). Pp. 30. (London: Applied Geochemistry Research Group, Imperial College of Science and Technology, 1973.) [2012]
- Roraima: Report of the 1971 British Expedition to Mount Roraima in Guyana, South America. By Adrian Warren. Pp. v+152. (Cobham, Surrey: Adrian Warren, Whiteoaks, Harebell Hill, 1973.) [2812]

### Other Countries

- Deutscher Wetterdienst. Deutsches Meteorologisches Jahrbuch, Bundesrepublik, 1970. Pp. xxxvi+242. (Offenbach a.M.: Deutscher Wetterdienst, 1973.) [1911]
- Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 2313: Nachweis und Reizbedingungen Olfaktorisch und Rhinosensibel Evozierter Hirnrindensummenpotentiale sowie Konzept einer klinischen Computer-Olfaktometrie. Von Dr. Claus Herberhold. Pp. 126. (Opladen: Westdeutscher Verlag, 1973.) 27.50 DM. [1911]
- International Union for Conservation of Nature and Natural Resources. IUCN Publications, New Series, No. 26: Planning for Man and Nature in National Parks—Reconciling Perpetuation and Use. By Richard R. Forster. Pp. 84. (Morges, Switzerland: International Union for Conservation of Nature and Natural Resources, 1973.) [1911]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 73-25: Electromagnetic Fields of Oscillating Magnetic Dipoles Placed Over a Multilayer Conducting Earth. By A. K. Sinha and L. S. Collett. Pp. 48. \$2. Paper 73-28: An Ionization Chamber for Continuous Monitoring of Atmospheric Radon—Radon-222 Levels in Ottawa and Gatineau Hills, Canada. By Willy Dyck. Pp. vi+11. \$2. Geological Map 1342A: Kashesibaw Lake, (East Half), Newfoundland-Quebec. (Ottawa: Information Canada, 1973.) [1911]
- European Organization for Nuclear Research-CERN. CERN 73-10: The Theory of Ionization Growth in Gases Under Pulsed and Static Fields. By A. J. Davies and C. J. Evans. Pp. x+111. (Geneva: CERN, 1973.) [1911]
- Bulletin of the American Museum of Natural History, Vol. 152, Article 4: A Revision of the Moth Genera *Neptrotæa* and *Chesiadodes* (Lepidoptera, Geometridae). By Frederick H. Rindge. Pp. 205-252. (New York: American Museum of Natural History, 1973.) \$2.85. [1911]
- Institut National de la Santé et de la Recherche Médicale. La Cyroconservation des Cellules Normales et Néoplasiques/ The Cryopreservation of Normal and Neoplastic Cells. (Proceedings of the International Conference, Villejuif, France, June 30, 1973.) Pp. 215. (Paris-Cedex: Institut National de la Santé et de la Recherche Médicale, 1973.) [1911]
- United States Department of the Interior: Geological Survey. Water-Supply Paper 2095: Quality of Surface Waters of the United States, 1968. Part 6: Missouri River Basin. Pp. xi+394. \$2.35. Water-Supply Paper 2110: Surface Water Supply of the United States, 1966-70. Part 3: Ohio River Basin. Vol. 4: Ohio River Basin Below Wabash River. Pp. ix+801. \$3.70. Water-Supply Paper 2115: Surface Water Supply of the United States 1966-70. Part 5: Hudson Bay and Upper Mississippi River Basins. Vol. 3: Upper Mississippi River Basin Below Keokuk, Iowa. Pp. viii+607. \$3.20. Water-Supply Paper 2125: Surface Water Supply of the United States, 1966-70. Part 9: Colorado River Basin. Vol. 2: Colorado River Basin From Green River to Compact Point. Pp. viii+634. \$32.00 (Washington, DC: Government Printing Office, 1973.) [1911]
- Reports of the Australian Academy of Science. No. 16: Symposium on Biological Memory. Pp. 77. (Canberra, ACT: Australian Academy of Science, 1973.) [2011]
- United States Department of the Interior: Geological Survey. Professional Paper 807: Geochemical Anomalies of a Claypit Area, Callaway County, Missouri, and Related Metabolic Imbalance in Beef Cattle. By Richard J. Ebens, James A. Erdman, G. L. Feder, Arthur A. Case and Lloyd A. Selby. Pp. iii+24. (Washington, DC: Government Printing Office, 1973.) 65 cents. [2011]
- Australian Academy of Science. Science and Industry Forum. Report No. 6: Industry and the Environment. Pp. 60. (Canberra, ACT: Australian Academy of Science, 1973.) [2011]
- Statistical Office of the European Communities. Basic Statistics of the Community: Comparison with some European Countries, Canada, the United States of America, Japan and the Union of Soviet Socialist Republics. Twelfth edition. Pp. 218. (Luxembourg: Statistical Office of the European Communities, P.O. Box 1907, 1973.) [2111]
- International Atomic Energy Agency, Vienna. Bibliographical Series No. 41: Isotope Techniques in Hydrology, Vol. II (1966-1971). Pp. 223. 190 schillings; £3.70; \$9. Computer Models and Application of the Sterile-Male Technique. (Proceedings of a Panel organized by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, held in Vienna, 13-17 December 1971. Panel Proceedings Series.) Pp. 192. 168 schillings; £3.30; \$8. (Vienna: IAEA; London: HMSO, 1973.) [2111]
- Australian Journal of Zoology. Supplement No. 22: The Sphaeroceridae (= Borroridae or Cypselidae; Diptera Cyclorhapha) of the Australian Region. By O. W. Richards. Pp. 297-401. (East Melbourne, Victoria: Editorial and Publications Service, CSIRO, 372 Albert Street, 1973.) [2111]
- Canada. Ministry of State—Science and Technology. A Research Study on Science Communication. By Orest Dubas and Lisa Martel. (Media Impact. Vol. 1: Interim Report.) Pp. 50. (Ottawa: Information Canada, 1973.) [2111]
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- Australia: Commonwealth Scientific and Industrial Research Organization. Twenty-fifth Annual Report, 1972/1973. Pp. 99. (East Melbourne, Victoria: CSIRO, P.O. Box 89, 1973.) [2311]
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